Visual Perception in Jumping Spiders (Araneae,Salticidae)

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CHRISTCHURCH NEW ZEALAND

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Abstract

Visual perception is the acquisition, organisation, identification, and interpretation of visual sensory information in order to represent and understand the environment. Classically considered a research field of psychology and philosophy, since the rise of sensory neuroscience, the study of perception has been adopted by biologists. The visual system of jumping spiders (Salticidae) is one of the most unique in the animal kingdom. This, in conjunction with their specialised hunting behaviours, small nervous system, willingness to respond to images on a screen, and often unique dietary preferences make salticids an exceptional model animal for studying visual perception. I used these and other unique features of salticids to shed new light on the process of visual perception.

Salticids have a pair of large forward-facing camera type eyes (known as primary eyes) which feature high resolution vision and have their retinae at the end of long, moveable, eye-tubes with which they continually scan their environment. Additionally, they have three pairs of smaller eyes that primarily act as motion detectors. These feature wide fields of view and collectively encompass a field of view of c. 360^{o}.

Taking advantage of the specialisation and unique dietary preferences of the east African jumping spider *Evarcha culicivora* I show that they perceive abstract stick-figures of *Anopheles* mosquitoes specifically as their preferred prey, even when the elements of the stick-figure are disconnected and rearranged. However, if the angles between the various elements are altered, the image is no longer categorised as a prey item. In contrast, another salticid, *Hypoblemum albovittatum*, a generalist predator, showed a lower affinity to the stick-figure images over more realistic digital stimuli. This work also showed potential effects of specialisation on perception, which seem to enable rapid recognition using low level cues by bypassing holistic, or gestalt processing.

Using a specialised eye-tracker to record the primary eye retinal movements while presenting the spiders with different digital stimuli, I classified some characteristics of the initial steps of the visual perception – specifically regarding retinal scanning. This work has shown that scanning motions are part of a closed loop system that follow the outlines of stimuli, rather than an independent and systematic to-and-fro protocol for the accumulation of visual information. Moreover, the scanning movements are strongly driven by the biological relevance of the stimulus and are subject to priming through the secondary eyes.

A further important aspect of visual perception is the perception of depth. There is little agreement on how salticids achieve depth perception. While several different processes have been suggested,

evidence for these has been somewhat elusive. The structure and location of their eyes gives the potential for utilisation of both binocular and/or monocular depth cues. In the first work investigating retinal movements in salticids, Mike Land[1] found no evidence of changes in the length of the eye-tubes of the primary eyes, which would correspond to a change in focal distance and thus accommodate depth judgments. This, coupled with the fact that the lenses of the eyes are part of the exoskeleton, suggests that salticids do not possess the ability to accommodate their eyes for depth vision. In recent work, Nagata et al.[2] suggested that the unique tiered retinal structure of the primary eyes enabled depth perception through a comparison of the amount of 'defocus' in the different layers due to chromatic aberration. In order to address the question of depth perception, I ran three experiments. In these, the use of binocular depth perception was ruled out as necessary for accurate depth perception. I also attempted to replicate one of the experiments run by Nagata et al.[2], but failed to achieve the same results. Finally, an eye-tracker was used to record the retinal scanning movements of jumping spiders while presenting them with a stimulus at different distances. Here, I found evidence that challenges the relied upon notion that salticids do not use 'accommodation' for depth perception. Overall, this work demonstrated that monocular cues are necessary and sufficient for depth perception, which requires the use of the primary eyes.

In this work, three different levels of visual perception have been investigated, from the initial processes of visual scanning, to the cognitive aspects of categorisation and object recognition and the perception of depth. I found evidence for different perceptual processes among predatory generalists and specialists, revealed the ability of jumping spiders to perceive abstract concepts, and uncovered new evidence for depth perception in salticids. I believe this work provides new perspectives on the perceptual capabilities of these amazing animals despite their minute nervous systems. Hopefully, my work will lead to novel and exciting research in spider vision, perception and arachnid neurobiology.

[1] **Land, M. F.** (1969a). Movements of the retinae of jumping spiders (Salticidae: Dendryphantinae) in relation to visual optics. *Journal of Experimental Biology* **51**, 471-493.

[2] **Nagata, T., Koyanagi, M., Tsukamoto, H., Saeki, S., Isono, K., Shichida, Y., Tokunaga, F., Kinoshita, M., Arikawa, K. and Terakita, A.** (2012). Depth Perception from Image Defocus in a Jumping Spider. *Science* **335**, 469-471.

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Preface

The data chapters (2-6) of this thesis were written as a collection of stand-alone papers for submission in peer-reviewed journals and Chapters 2 and 3 have already been published (see below). It is due to this format that some repetition between the chapters, mainly in the introduction, was unavoidable. Chapter 6 is co-authored by Dr. Ximena Nelson, who carried out the 'flying saucer' experiments. I analysed that section and carried out the rest of the behavioural experiments as well as the eye-tracker work and wrote the paper. In all other chapters, I developed the experimental design, carried out the experiments, analysis and writing. All submitted chapters are unaltered except to fit thesis style.

Published:

Chapter 2**: Dolev, Y. and Nelson, X. J.** (2014). Innate pattern recognition and categorization in a jumping spider. *PLoS ONE* **9**, e97819. * Appendix 4

Chapter 3: **Dolev, Y. and Nelson, X.** (2016). Biological relevance affects object recognition in jumping spiders. *New Zealand Journal of Zoology* **43**, 42-53. * Appendix 5

In preparation

Chapter 6: Dolev, Y, Harland D. P., O'Carroll D. C. and Nelson X. J. Out of its depth: A reassessment of depth judgements in jumping spiders (Araneae, Salticidae)

“All our knowledge has its origins in our perceptions.”

Leonardo da Vinci

Chapter 1

Introduction

While seemingly an effortless process, vision is actually an amazingly complex task. From the intricate structures of the photoreceptors and the retina, through the scanning of the visual environment, to the extraction of its basic structure and elements ultimately leading to recognition, visual processing is the ability of interpreting the surrounding environment through the processing of information that is contained in visible light. When contemplating animal vision and object recognition, vertebrate vision is the model most commonly thought of. However, the vertebrate design is only one of at least 10 distinct solutions natural selection has found for enabling animals to visually negotiate their worlds (Land and Nilsson 2012). Each eye design reflects compromises in achieving diverse goals, from detection of objects under different ambient light levels, to perception of movement and seeing detailed characteristics of objects. Some animals, ourselves included, can house eyes large enough to accommodate these competing tasks while allowing for large brains that can handle the massive neural processing required to make sense of the input from these eyes. However, small animals face trade-offs in eye design resulting in an impressive array of very different eye designs (reviewed in Land 2012).

Perception is the acquisition, organisation, identification, and interpretation of sensory information in order to represent and understand the environment (Schacter 2011) in a biologocally-relevant manner. Throughout the animal kingdom, all perception stems from neuronal afferent signals which are the result of stimulation of the various sense organs (Kandel et al. 2000). In the case of hearing, pressure waves traveling in the air (or water) stimulate specialised hair cells in the ears, triggering afferent signals. In smell, it is mediated by odour molecules. In vision, photons of light strike photoreceptors located in the retina of the eyes, and these transduce the electromagnetic energy into neural signals, which in turn are transmitted to the central nervous system (CNS) for further processing (Kandel et al. 2000, Goldstein 2009). Contrary to our intuitive understanding based on personal experience, our perceptions are not direct copies of the world around us. Rather, they are a representation of external events created through the functional anatomy of the CNS and the molecular dynamics of populations of nerve cells therein (Kandel et al. 2000). Each sensory modality is mediated by a distinct neural system, and to understand that sensory modality, it is important to know what, and how, each component of a sensory system contributes to perception.

Perhaps because we are primarily visual animals, perception in the visual modality is the most thoroughly investigated of the senses. This is exemplified by the results of searching for papers by topic in

the website Web of Science (©2016 Thomson Reuters). Searching for the key words 'visual perception' with no filters resulted in 54,597 results. The same search using the key words 'auditory perception' yielded 16,159 results. Searching using the key words 'olfactory perception' yielded just 2865 results. Finally searching for either 'mechano perception', mechanosensory perception' or 'mechanoreception perception' each yielded less than 150 results.

Early thinking about visual perception was greatly influenced by the British empiricist philosophers, notably John Locke and George Berkeley, who thought of perception as an atomistic process whereby simple sensory elements, such as colour, shape, and brightness, were assembled in an additive way, component by component (Berkeley 1709, Locke 1841). The modern view, that perception is not atomistic but holistic, was first emphasised in the early twentieth century by the German psychologists Max Wertheimer, Kurt K*ö*ffka and Wolfgang Köhler, who founded the school of Gestalt psychology (Wertheimer 1923, Köhler 1944). This view states that visual perception is an active and creative process that involves more than just the information provided to the retina by any given stimulus element. The central idea of Gestalt psychologists is that the perceptual interpretation made of any visual object depends not just on the properties of that element, but also on its contextual interactions. To this, as biologist, we might also add the natural history of the perceiver (Dukas 1999).

Since the rise of sensory neuroscience, great advances have been made in our understanding of visual systems. For example, there is now a fairly complete understanding of how photons are transduced by photoreceptors in the retina into electrical activity (Yarfitz and Hurley 1994), how (primarily primates) the retina processes this activity in parallel pathways (Lennie 1980, Srinivasan and Dvorak 1980, Steller et al. 1987, Krubitzer and Kaas 1989, El Jundi and Homberg 2010) and we even have some insight into how visual information is processed at the cellular level (reviewed in Kandel et al. 2000). While the processes of a transducing an external stimulus into neural activity is largely a passive process (Gregory and Zangwill 1987), perception as a whole is anything but a passive receipt of these signals. Rather, perception is strongly shaped by the animals' life history, genetics, learning, memory, expectation, and even attention (Dukas 1998, 2004 Goldstein 2009). Two well documented examples of such effects are habituation and priming.

Habituation (Dong and Clayton 2009) is a form of non-associative learning which is manifested as a decreased response to a repeated stimulus (Mazur 2015). Among its benefits, habituation enables an animal to distinguish biologically 'irrelevant' background stimuli, such as perhaps the shadow of leaves moving in the wind, from biologically important stimuli, such as predator, prey or mate detection amid that

background, while also preventing the organisms' nervous system from being flooded with irrelevant information (Klingner et al. 2014).

Priming is an implicit memory effect in which exposure to one stimulus (i.e., the primer, which can be in any sensory modality) influences the response to a subsequent stimulus (Wiggs and Martin 1998), whether in the same or another sensory modality (cross-modaltity priming), by altering what the subject perceives. The effects of priming on perception in humans are so well documented that the $600 billion industry of advertising relies on it (Yi 1990, Campbell and Kirmani 2000, Dijksterhuis et al. 2005). A classic (and slightly more scientific) example of how motivational priming can affect visual perception in humans is documented in a study by Balcetis & Dunning (2006). In this study, participants were shows a series of numbers and letters and were told to perfume a task according to the image (one task for numbers, another for letters). One of the images was of an ambiguous figure which could be perceived as either the letter 'B' or the number '13'. The participants perceived (according to their own reports and confirmed by eye tracking data) the ambiguous figure as the letter B or the number 13 according to which one assigned them with outcomes they favoured.

Sensory systems are expensive both in terms of their neuronal 'real-estate' (in primates, for example, the visual cortex alone is estimated to take up almost 30% of the entire cortex, Kendal et al. 2000) and in terms of the metabolic cost of their maintenance (Aiello and Wheeler 1995, Laughlin et al. 1998, Moran et al. 2015). Indeed, in humans it is estimated that the brain accounts for 20% of resting oxygen consumption and between 20%-25% of the resting metabolic rate (Aiello and Wheeler 1995, Clarke and Sokoloff 1999, Laughlin 2001) and, in one species of fish, vision alone accounts for 15% of its resting metabolic rate (Moran et al. 2015). Indeed, comparative studies suggest that of the limiting factors for the size of primate brains is the metabolic expense of maintaining the brain throughout life (Aiello and Wheeler 1995), or the energetic demands on pregnant mothers resulting from the developing brain of their foetus (Martin 1996). It is therefore reasonable to assume the sensory systems are under constant evolutionary pressure and that they are highly tuned to maximise an organism's fitness. Failing that, these senses would rapidly 'de'-evolve (Moran et al. 2015). Salticids represent a fascinating demonstration of the trade-offs between a highly tuned visual system and, with brains the size of a poppy seed (Jackson and Harland 2009, Menda et al. 2014), minimal neuronal 'real-estate'. As a result, salticids have evolved one of the most remarkable visual systems. In this thesis I address various aspects of the visual perception in salticids.

Jumping spiders are a well-studied group of animals. As their name implies, the spiders in this family, Salticidae, jump to escape from their enemies and to pounce on their prey once they have stalked

it, solitarily and quietly, leopard-like (Harland and Jackson 2000). Typically measuring less than 10 mm in body length, salticids have a surprisingly small nervous system, containing roughly 500,000 neurons (Land M. pers. comm. with XJN), or half those of a cockroach (Strausfeld 1976, Burrows 1996). Despite this, salticids are renowned for their visually-guided predatory behaviours that are strikingly complex and flexible (Jackson and Wilcox 1993, Wilcox et al. 1996, Tarsitano and Jackson 1997, Tarsitano and Andrew 1999, Harland and Jackson 2004). Most salticids stealthily approach targeted prey to within 2-3 cm, whereupon they pounce on it. Different salticid species sometimes specialise on catching specific types of prey. For example, *Portia fimbriata* has a specialised predatory strategy for preying on other salticids (Harland and Jackson, 2004), while *Evarcha culicivora* specialises at preying on blood-fed female mosquitoes in the genus *Anopheles* (Nelson et al. 2005, Nelson and Jackson 2006). In many instances, these specialisations manifest themselves in very obvious prey-specific hunting behaviours (Jackson and Blest 1982, Harland and Jackson 2004, Nelson et al. 2005, Harland and Jackson 2006). These behaviours are so specific to the preferred prey that, in essence, they communicate what it is that the salticid perceives, as execution of these behaviours by the spiders towards a given stimulus, it tells me that the salticid has identified that stimulus as its preferred prey.

However, it is neither their jumping ability nor the unique predatory behaviours of salticids that commands attention so much as their outstanding vision. The eyes of most spiders lack the structural complexity required for acute vision (Homann 1971, Land and Nilsson 2012), but the unique, complex eyes of salticids support resolution ability with no known parallel in other animals of comparable size (Land 1969a, Land 1969b, Williams and McIntyre 1980, Blest et al. 1990). Unlike other spider families, the eyes of salticids are arranged in a single row that extends around the prosoma. The salticid visual system is a distributed visual system, comprised of four pairs of simple (camera-type) eyes. In addition to a single, large, forward-facing pair of eyes (principal or Anterior Median (AM) eyes), these are three smaller pairs of eyes: the forward-facing anterior lateral (AL) eyes, the lateral-facing posterior median (PM; reduced in most species), and the rear-facing posterior lateral (PL) eyes (Land 1985). These latter three pairs of eyes (AL, PM, PL) are collectively known as the 'secondary eyes' and jointly encompass c. 360^{o} field of view, with considerable binocular overlap in the fields of view of the more forward-facing AL eyes, but only minimal overlap between the AL and PL eyes (see Appendix I, Figure S1; Land 1971, Land 1972, Zurek et al. 2010, Zurek and Nelson 2012). The cornea of all eyes are fixed to the carapace and the retinae of the secondary eyes have no freedom of motion. While the AL eyes possess a fovea and convey some additional spatial information (Forster 1979, O'Carroll 1989, Zurek et al. 2010, Zurek and Nelson 2012), collectively the secondary eyes largely act as motion detectors, with movement detected in their fields of view causing a

rapid optomotor response which results in the target being acquired by the AM eyes (Land 1971, Forster 1979). The AM eyes have a narrow boomerang-shaped retina, subtending about 20° vertically by 1° horizontally in the central region, which is roughly six receptor rows wide (Land 1972). These eyes provide outstanding spatial acuity (as low as 0.04°) over a narrow field of view of 3-5° (Land 1969a, Land 1969b, Williams and McIntyre 1980, Blest et al. 1990).

The small field of view of the AM eyes is overcome through a specialised arrangement of muscles (Land 1969a) which surround the eye-tubes. These muscles extend the visual field of the AM eyes to about 35° on either side of the body axis (Land 1972) by allowing motion of the the eye-tubes in 3 dimensions. Four different types of retinal motion in the salticid primary eyes were initially described by Land (1969a) through the use of a specialised ophthalmoscope to look into the retinae of salticid AM eyes. These are 'spontaneous activity', 'saccadic motions', 'tracking motions' and 'scanning motions' (Land 1969a). Land (1969a) described the various retinal movements as comprised of two primary types: rotational movements and translational (to-and-fro) movements. Of these, 'scanning' is the most relevant for visual perception, as it appears to have a primary role in object recognition.

Land (1969a) proposed that scanning functioned as a search by the eyes for lines situated at specific locations on the object being viewed; the salticid's objective being to distinguish mates and conspecific rivals from prey. However, salticids often adopt intricate classification schemes for potential prey, mates and rivals (Jackson and Blest 1982, Harland and Jackson 2004, Nelson et al. 2005, Harland and Jackson 2006). Hence Land's (1969a) original hypothesis needs to be modified to include the salticid's entire repertoire of rotation-translation routines. Instead of any one stereotypical rotation-translation routine, it is reasenable to predict different routines for identifying different objects. Given the the field of view of the AM eyes is overlaped by that of the AL eyes, and that the spacial acuity of the AL eyes rivals that of the best compound eyes of insects (Land and Nilsson 2012), salticids may, in fact, guide the movements of their AM eye retinae using the AL eyes. According to this hypothesis, salticids use the minute fovea of their AM eyes and their highly mobile eye tubes to build a 'high resolution' picture of what is being looked at by the AL eyes (Zurek 2012). By extension, and given the resolution of the posterior-median (PM) eyes (Land 1971, Hardie and Duelli, 1978, Zurek and Nelson 2012), it may be that information from the rear visual world is 'communicated' to the forward-facing AM eyes, which alongside the spatially acute AL eyes, can direct movements of the AM eye-tubes to facilitate visual searches for specific cues which may aid in rapidly assessing specific objects. For example, the PM eyes might see something 'mosquito-like' and communicate that to the AM eyes. The spider, by this stage has performed its optomotor response and will be facing the object, which will be seen by the more spatially acute AL eyes.

Simultanously, the AM eyes will be scanning for specific aspects that might desgnate the object as a mosquito (for example, the angles between the legs and the body, see Chapters 2 and 3). Feedback from the AL eyes to the AM eyes might help refine 'where to look'. Thus a complex set of feedback loops might be used by salticids to speed up object identification using the very spatially acute fovea of the AM eyes. This might be needed because of the tiny field of view of the fovea, which would make scanning sequentially a long process which may well results in death, if the object were, for example a wasp rather than a mosquito.

In this thesis, I examine various aspects of salticid visual perception in relation to the life histories and predatory behaviours of differnet species. Two main approaches are taken. The first (Chapters 2-3) is classic animal behaviour experiments using choice tests to investigate how differents salticid speceis perceive different abstract images. For this, I exploit their unique predatory behaviours as indicators of their visual processes and decsion-making regarding object identification. The second approach taken (Chapters 4-5) is a specialised salticid eye-tracker (see Appendix 1) which allows viewing and recording the retinal movements of the AM eyes while presenting different stimuli to to either the primary and/or secondary eyes. Finaly, in Chapter 6, both of these approaches were employed in a series of experiments to investigate a long-standing question in salticid vision, namely how they perceive depth, which is so fundamental to their hunting success as jumping spiders.

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Chapter 2

Innate pattern recognition and categorisation in a jumping spider

Some of the stimuli used in this chapter (Created by Y. Dolev)

Dolev, Y. and Nelson, X. J. (2014). Innate pattern recognition and categorization in a jumping spider. *PLoS ONE* **9**, e97819. doi:10.1371/journal.pone.0097819 * Appendix 4

Abstract

The East African jumping spider *Evarcha culicivora* feeds indirectly on vertebrate blood by preferentially preying upon blood-fed *Anopheles* mosquitoes, the vectors of human malaria, using the distinct resting posture and engorged abdomen characteristic of these specific prey as key elements for their recognition. To understand perceptual categorization of objects by these spiders, we investigated their predatory behaviour toward different digital stimuli - abstract 'stick figure' representations of *Anopheles* constructed solely by known key identification elements, disarranged versions of these, as well as non-prey items and detailed images of alternative prey. We hypothesised that the abstract images representing *Anopheles* would be perceived as potential prey, and would be preferred to those of non-preferred prey. Spiders perceived the abstract stick figures of *Anopheles* specifically as their preferred prey, attacking them significantly more often than non-preferred prey, even when the comprising elements of the *Anopheles* stick figures were disarranged and disconnected from each other. However, if the relative angles between the elements of the disconnected stick figures of *Anopheles* were altered, the otherwise identical set of elements was no longer perceived as prey. These data show that *E. culicivora* is capable of making discriminations based on abstract concepts, such as the hypothetical angle formed by discontinuous elements. It is this inter-element angle rather than resting posture that is important for correct identification of *Anopheles*. Our results provide a glimpse of the underlying processes of object recognition in animals with minute brains, and suggest that these spiders use a local processing approach for object recognition, rather than a holistic or global approach. This study provides an excellent basis for a comparative analysis on feature extraction and detection by animals as diverse as bees and mammals.

Introduction

Object recognition is the ability to perceive the physical properties (such as shape, colour and texture) of an object and apply semantic/cognitive attributes to the object (Enns 2004), such as an understanding of its use, or classification of the object as prey, predator or irrelevant. The process leading to recognition is typically, though not exclusively, viewed as a bottom-up hierarchy in which information is processed sequentially with increasing complexity. In vertebrates, the idea is that lower-level cortical processors, such as the primary visual cortex, process the basic object components such as colour, depth and form, while higher-level cortical processors, such as the inferotemporal cortex in humans, are ultimately responsible for recognition (Bar 2003). Historically, perhaps one of the best-known attempts at explaining perception and recognition is that of Gestalt psychology.

The central tenet of Gestalt psychology is that the whole differs from the sum of its parts. The theoretical framework underlying Gestalt ideas is holism, which states that systems and their properties should be viewed as wholes, not as collections of parts (Wagemans et al. 2012). This contrasts with earlier structuralist hypotheses, which state that perceptions can be derived by identifying the elementary parts (Titchener 1909, Rock and Palmer 1990, Förster and Higgins 2005). Modern research into visual processing has changed its focus from gestaltism vs. structuralism to global vs. local processing (Kimchi 1992, Förster and Higgins 2005), with an expanded focus from the psychological processes of perception to include physiological processes (Heinze et al. 1998). The global processing framework results in the notion that an object is recognised only when its elements form the whole image, while the local processing framework requires the identification of correct elements, points and edges, but not necessarily the image as a whole. This distinction also suggests potential differences in the neurobiological processes underlying object recognition (Heinze et al. 1998).

For a predator that relies on vision, the ability to classify an object as predator or prey will be under strong selection. However, the extent to which visual predators further classify items can vary considerably. Some predators make rapid decisions and do minimal classifying of prey into particular types, relying instead on key features, such as seeing an object of a specific size range moving in a specific orientation, as identifiers of prey (Barlow 1953, Lettvin et al. 1959, Ewert 1997,2004). Examples of this approach can be found among amphibians (Ingle 1983, Ewert 2004) and mantises (Prete et al. 2011), which adopt remarkably similar approaches despite possessing very different nervous systems. Many jumping spiders (Salticidae) also rapidly categorise objects as prey or non-prey based on only a few key features (Drees 1952, Forster 1985, Bednarski et al. 2012, Spano et al. 2012). However, it is also amongst the

salticids that some of the most precise prey identification and prey preference behaviours among animals is found.

An extreme case of such preference is that of *Evarcha culicivora*. Uniquely, this East African salticid feeds indirectly on vertebrate blood by selectively preying upon female mosquitoes (particularly *Anopheles*, famous as the vectors of malaria) that have recently fed on blood. These spiders are capable of using vision alone to discriminate between their preferred prey, blood-fed female *Anopheles*, and similar looking male *Anopheles*, female *Anopheles* that have not fed on blood, non-anopheline mosquitoes, as well as various similar-sized non-mosquito prey (Jackson et al. 2005, Nelson and Jackson 2006, Jackson and Nelson 2012). These experiments have also shown that for correct identification *E. culicivora* uses a complex non-linear process involving specific elements of the prey, including an engorged abdomen, resting posture and antennae (Nelson and Jackson 2006, Nelson and Jackson 2012).

Like other salticids, *E. culicivora* has exceptional eyesight, which is used to locate, stalk and finally pounce on its prey (Jackson and Pollard 1996). Salticids have large forward-facing principal eyes that are specialised for high resolution vision but within a very narrow (ca. $<5^{o}$) field of view (Land 1969a, Land 1969b, Williams and McIntyre 1980, Land 1985, Blest et al. 1990) which is compensated for with complex movements that scan up to ca. 28^{o} to either side of the body axis (Land 1969a). Additionally, salticids have three pairs of motion-sensitive secondary eyes with wide fields of view and which collectively encompass up to 360^{o} (Land 1971, Land 1972, Zurek et al. 2010, Zurek and Nelson 2012).

E. culicivora's unique dietary preferences, which can be expressed using vision as the sole sensory modality for prey classification (Nelson and Jackson 2006, Nelson and Jackson 2012), make this species an excellent subject for the study of recognition and classification of prey. Here we presented the spiders with abstract representations of potential prey ('stick figures') differing in their level of simplicity to determine whether predatory behaviour and prey classification was elicited by biologically unrealistic prey containing only key elements (local processing). Stimuli included stick figures of *Anopheles* mosquitoes in their resting posture, as well as non-prey items and alternative prey items. We used single-choice predatory behaviour experiments to determine whether or not *E. culicivora* 'viewed' abstract representations of prey as potential prey, and two-choice predatory behaviour experiments to test for specific preference between stimuli. Due to *E. culicivora*'s known ability to discern specific elements of prey, we predicted that these specialised visual hunters would stalk and pounce on abstract representations of prey. We also predicted that *E. culicivora* would choose simplified representations of its preferred prey over realistic images of alternative non-preferred prey, showing that it categorises these images as its preferred prey item.

Methods

(a) *General*

All spiders used in this study were at least second generation laboratory reared *E. culicivora* individuals, and no juveniles tested had ever encountered mosquitos. Testing was carried between 0730 and 1200 h in a temperature-controlled laboratory set to 24^{o} with a photoperiod of 12L:12D, lights on at 07:00. Test spiders were unmated adults (body length, 4.5-5.5 mm) and juveniles (1.5-2.5 mm). Standard rearing and maintenance was as in earlier studies (for details, see Jackson et al. 2005, Nelson and Jackson 2006). Spiders were caged individually and were fed to satiation once a week on Drosophila spp. Two h prior to their use as prey, Drosophila were given a honey and human blood (obtained from a blood bank) meal by inserting a cotton dental wick dipped in the mixture into their rearing container. Test spider hunger levels were standardised by a 5-7 day pre-trial fast. Test spider predatory behaviours (noticing, stalking and/or pouncing) and their timing were recorded during all experiments. Noticing behaviour is characterised by the spider performing an optomotor response to face the stimulus with its AM eyes and subsequently staring continuously at the stimulus for a few seconds. Stalking behaviour is characterised by the salticid slowly stepping toward the prey while visually fixated on the prey. Both are reliably identifiable behaviours.

(b) *Stimuli*

Stimuli consisted of videos of repeated sporadic movement of different images (Table 1), created using Adobe Photoshop CS5 in greyscale. Image 1 was a realistic line drawing of a blood-fed female *Anopheles gambiae* mosquito in typical resting posture, while the simplified images 2 (not blood-fed) and 3 (blood-fed) were similar but used only straight lines and ovals, with the latter depicting a blood-fed mosquito with an engorged abdomen, known as an important prey-identification cue (Nelson and Jackson 2012). Image 4 was a disarranged version of image 3, created so as to not alter the respective angles of any of the elements of image 3, while ensuring the elements were disconnected and, to humans, no longer resembling a mosquito. Image 5 was created by rendering a photograph of a housefly (*Musca domestica*) to greyscale and removing the background. Image 6, a circle the size of a housefly was created as a control, as were images 7 and 8. Image 7 was an altered version of image 4 where the angles of each of the elements of the image were altered and image 8 was a disarranged version of image 6, broken into 4 unequal sections. All images were created on a background of 250,250,250 RGB and had black pixel counts

between ca. 200 and 550 pixels (Table 1). Screen size was set to 1024 X 768 pixels. All images were sized similarly and were presented at biologically relevant sizes (to the nearest 0.5 mm).

To create the stimuli, one (in single-choice predatory behaviour experiments) or two (in two-choice predatory behaviour experiments) images were rendered into videos of repeated, horizontal (single-choice predatory behaviour experiments) or vertical (two-choice predatory behaviour experiments) motion (two bouts of back and forth movement every 10 s). Motion speed was 9^{o}/s, at a viewing distance of 10 cm, and movement distance was set to be 8^{o} visual angle. These parameters were selected to maximise the attention of the spiders (Zurek et al. 2010, Zurek and Nelson 2012) (see link to video S1 in Appendix 1 for a sample stimulus video).

Table 1: Images (with the numbering as referred to in the text) and the parameters of stimuli used as stimuli in both experiments. Relative contrast is the number of black pixels in the frame. Images 1–4 are based on *Anopheles* mosquitoes. 1 is based on King et al. (1943). Image 4 is a disarranged version of image 3. Image 8 is a disarranged version of image 6. Image 7 is based on image 4 where the angles of the various elements have been altered.

Stimulus	**1***	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Image								
Relative Contrast	250	230	518	545	500	211	545	211
Width (mm)	7	6	6	6	7	6	7	7

*Based on (King et al. 1943).

Videos were projected onto a screen using an AAXA M2 Micro Projector connected to a computer, and placed 100 mm from the screen. The videos were played on a continuous loop using VLC player software. The screen was made of two protective sheets of glass (each 2 mm thick, 5 cm wide X 5 cm long) with LCD screen polarisers from a Toshiba Tecra A9 PTS52C-MH409C laptop cut to size between them. This setup was used as we have found that the screen polarisers effectively reduce the brightness of the projected videos and did not result in a polarised image, while the glass sheets prevented the screen polarisers from getting damaged while being handled and cleaned. Due to the high spatial resolution of salticid principal eyes (ca. 11 minutes of arc, Land 1969b) images projected directly onto a screen will appear pixelated once the spider gets close. To overcome this, while maintaining life size images at high resolution, larger than life size stimuli were back-projected through a lens placed between the projector and a screen, which reduced the projected image by ca. a factor of 10. Fine tuning the size of the projected stimuli was achieved by varying the size of the VLC player window on the computer monitor.

(c) *Do jumping spiders view abstract images of prey elements as prey?*

To answer this question, we tested the predatory responses of the spiders to individual stimuli (single-choice predatory behaviour experiment). An angled wooden ramp supported by a wooden pole glued to a wooden base was placed in front of the screen and projector (see Figure 1A for dimensions). The apparatus was painted with two coats of polyurethane, but the top face of the ramp had a sticker marked with a 5 mm grid to allow accurate measurement of the spider's distance from the stimulus when a particular

behaviour was observed. The ramp was wiped with 80% ethanol and allowed to dry for 15 min between each test to eliminate possible chemotactile traces from spiders in previous tests.

For each test, a spider was placed on the ramp and covered with a petri dish, at a distance of 6 (juveniles) or 10 (adults) cm from the centre of the petri dish to the screen. These distances were used as they are far enough from the screen so that the spiders couldn't 'walk' directly onto the stimuli, while being close enough to enhance the chances of the spiders reacting to the stimuli (juveniles were less responsive to stimuli at a distance compared to adults). The screen was covered with a piece of black cardboard until test spiders were released to prevent them seeing the stimulus until tests began. Once the spiders were relaxed (staying stationary or grooming) the screen was uncovered, the petri dish was removed and timing started. Tests ended when the spiders pounced on an image or walked/jumped off the ramp. If a spider noticed the image, the session was considered successful and tests were not repeated with the same spider. A spider that failed to notice the stimulus was tested up to twice in one day, or up to a total of 4 times in the following 3 days.

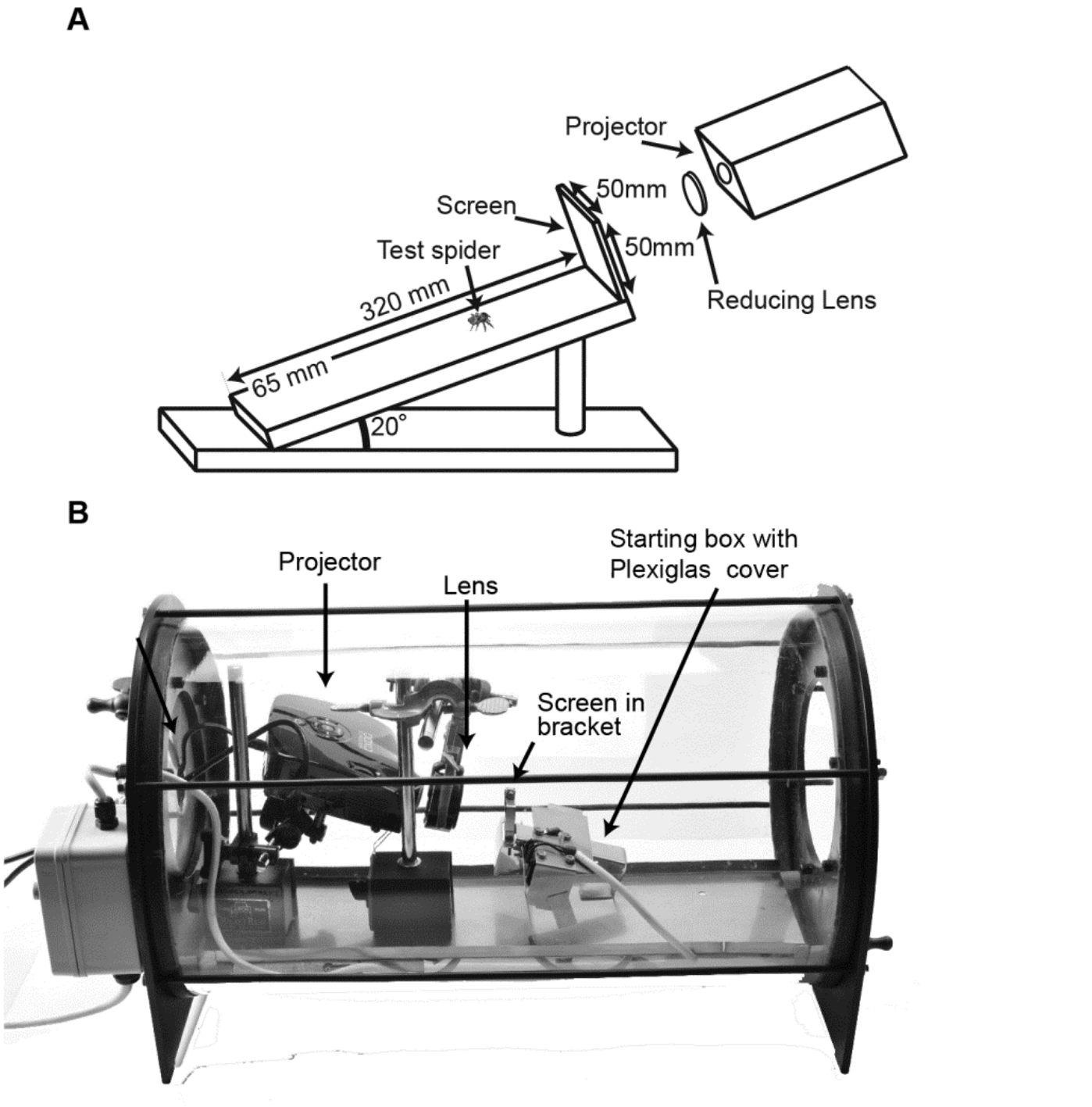

Figure 1: Experimental apparatuses used.
A) Apparatus used in single-choice predatory behaviour experiment. Spiders (not to scale) were placed either 10 cm (adults) or 6 cm (juveniles) away from stimulus screen, and behaviour recorded. B) Apparatus used in the two-choice predatory behaviour experiment. Projector and reducing lens placed inside glass chamber 100 mm from screen and ramp complex.

(d) *Do jumping spiders view abstract images as their preferred prey?*

In this experiment we relied on *E. culicivora*'s proven preference for *Anopheles* mosquitoes and presented them with a two-choice test. All spiders used in this test were laboratory reared and had no prior experience with mosquitoes. For these tests, rendered movies contained two images (Table 3) which moved identically and simultaneously. In each test, which image was on the right and which was on the left was randomised. The movies were projected as above, but experiments were held within a specialised apparatus containing a stainless steel ramp (15 mm wide X 150 mm long; angled up by 25°) in front of the screen. The ramp was inside a glass chamber (diameter 300 mm, length 525 mm long) with removable sealing steel end plates (diameter 200 mm, kept off during this set of experiments). Welded to the ramp was a bracket onto which the screen was attached with a gap of 5 mm from the ramp. The ramp/screen unit ('ramp complex') sat mounted within holes on a stainless steel platform spanning the length of the cylinder (Figure 1b). In this way it could be removed for cleaning with 80% ethanol after each test and returned to the same place, while ensuring that the distance between the screen and the reducing lens and projector was always the same (and thus stimulus size was constant).

At a distance of 22 mm from the end of the ramp, a stainless steel 'starting box' (11 mm wide X 19 mm high X 22 mm deep; i.e., furthest point 44 mm from top end of ramp) was welded to the ramp complex (Figure 1B). The box had a transparent Plexiglas cover wired to an external controller so that it could be opened remotely. The spider was placed into the starting box and the door was closed. After 2 min, the ramp complex was put in place. Once the spider was away from the door of the starting box, after ca. 20 s, the door was opened and tests began. Tests ended with the spider pouncing on one of the two images on the screen or to jumping/walking off the ramp. Failing these two conditions, tests were stopped after 15 min. In this experiment we were interested in pouncing behaviour rather than in stalking behaviour, as the former constitutes a more distinct choice by the spiders. For this reason, both adult and juvenile spiders were released a short distance from the screen (see link to video S2 in Appendix 1 for a sample of the spider behaviour in this experiment).

(e) *Data analysis*

All analyses were done using SPSS Statistics v.20. For the single-choice predatory behaviour experiment, GLM analyses were performed to check for the main effects of stimulus type, 'sex', relative contrast (number of black pixels against a white background) which was either in the ca. 200 or ca. 500 pixels) and their interaction on the spider's choice to stalk the stimuli. Interactions between stimulus relative contrast and stimulus type were not analysed, as these are nested. Sexes were divided into three – female, male and juvenile as their sex cannot be discerned and their behaviour differs (Nelson et al. 2005, Nelson and Jackson 2012). In this model the dispersion parameter was set at 1, and type III sums of squares were used, though there was no qualitative difference from type I. Kruskal-Wallis tests were used to compare the predatory responses between the different sexes, with Mann-Whitney U tests for pairwise analysis. Cochran's Q tests were used to test how the different stimuli affected the chances of the spiders noticing the stimulus and the propensity to stalk and pounce. Friedman tests were used to test the effects of the different stimuli on stalking initiation distance, as well as their effects on the amount of time it took the spiders to start stalking. When these effects were found to be significant, McNemar tests were used for pairwise comparisons. For the two-choice predatory behaviour experiments, Binomial tests were used to test the spider's choices, as well as possible side-bias.

Results

(a) *Do jumping spiders view abstract images of prey elements as prey?*

A total of 195 successful sessions were run in the single-choice predatory behaviour experiment: 85 with adult females, 50 with adult males and 60 with juvenile spiders. When spiders initiated stalking behaviour, this almost always resulted in pouncing on the abstract prey (Appendix 1 Table S1). The type of stimulus had no effect on whether the spiders noticed it ($\chi2 = 6.71$, df = 6, $p = 0.349$, Appendix 1 Table S2). Stimulus type did affect the propensity to stalk the prey once it was noticed ($\chi2 = 37.87$, df = 6, $p < 0.001$), but did not affect the amount of time it took the spiders to 'decide' to stalk the prey (time between the spider first noticing the stimulus and initiation of stalking behaviour; $\chi2 = 3.928$, df = 6; $p = 0.686$, Appendix 1 Table S2). Once stalking was initiated, stimulus type had no effect on the propensity to pounce ($\chi2 = 4$, df = 6, $p = 0.677$, Appendix 1 Table S2). Stalking was therefore considered to be a true sign of predatory behaviour by the spiders. The spiders stalked the abstract images of mosquitoes (stimuli 1, 2, 3 and 4) significantly more often than the images of non-prey items (stimuli 6 and 7; Table 2). However, while the image of the fly

(stimulus 5) was stalked significantly more often than the altered, disarranged abstract image of the blood-fed mosquito (stimulus 7), it wasn't stalked more often than the image of the circle (stimulus 6).

GLMs on the propensity to stalk showed significant main effects of stimulus type ($\chi2 = 22.315$, $df = 6$, $p < 0.005$) and spider sex ($\chi2 = 7.413$, $df = 2$, $p < 0.05$), but not their interaction ($\chi2 = 9.270$, $df = 11$, $p = 0.597$). The effects of the relative contrast of the stimuli or its interaction with spider sex were also not significant (respectively, $\chi2 = 0.039$, $df = 1$, $p = 0.843$; $\chi2 = 0.431$, $df = 2$, $p = 0.806$). Females and juveniles were more prone to stalk stimuli (56.5% of 85 and 70% of 60 respectively) than males (38% of 50; females vs males: $U = 1732.5$, $p < 0.05$; juveniles vs males: $U = 1020$, $p < 0.001$, Mann-Whitney U test, Appendix 1, Tables S9, S11), while there was no significant difference between females and juveniles ($U = 2205$, $p = 0.099$, Mann-Whitney U test, Appendix 1, Table S11). Similarly, stimulus type had a significant effect on the propensity of females and juveniles to stalk (respectively: Cochran's $Q = 14.195$, $p < 0.05$; Cochran's $Q = 14.261$, $p < 0.01$, Appendix 1, Tables S3, S4, S7, S8) but not on that of the males (Cochran's $Q = 5.636$, $p = 0.465$, Appendix 1, Tables S5, S6). While there were no significant differences in how often the different sexes noticed different stimuli ($\chi2 = 5.762$, $df = 2$, $p = 0.056$, Kruskal-Wallis test, Appendix 1, Table S10), there were significant differences in the distances at which the they noticed the stimuli ($\chi2 = 14.021$, $df = 2$, $p < 0.005$, Kruskal-Wallis test, Appendix 1, Tables S10), with the females noticing the stimuli from significantly further away than males or juveniles (respectively, $U = 1471$, $p < 0.005$; $U = 1751$, $p < 0.0.005$, Mann-Whitney U tests, Appendix 1, Table S11). There were also significant differences between the sexes in their propensity to pounce once stalking was initiated ($\chi2 = 10.461$, $p < 0.01$, Kruskal-Wallis test, Appendix 1, Tables S9, S10), with the males less prone to pounce on prey than either females or juveniles (respectively: $U = 306.5$, $p < 0.01$; $U = 267$, $p < 0.01$, Mann-Whitney U tests, Appendix 1, Table S11). See Appendix 1, Tables S1-S11 for the full datasets.

Table 2: Responses and statistical comparisons of the spiders to the different stimuli**.** Note all stimulus sizes are equivalent, see Table 1. Upper section of the table contains the number of spiders that noticed the stimuli (N) and the percentage of stalking instances. The bottom section of the table contains the crosswise comparisons of the stalking responses to the different stimuli, using McNemar tests with a binomial distribution.

	1	**2**	**3**	**4**	**5**	**6**	**7**
							
N	27	28	27	30	29	27	27
Stalk (%)	74	64	74	77	56	33	24
	-	0.508	1	1	0.267	<0.001	<0.001
	-	-	0.549	0.549	0.774	<0.05	<0.005
	-	-	-	1	0.267	<0.05	<0.001
	-	-	-	-	0.302	<0.005	<0.001
	-	-	-	-	-	0.118	<0.05
	-	-	-	-	-	-	0.219

(b) *Do jumping spiders view abstract images as their preferred prey?*

A total of 123 successful sessions were run in the two-choice predatory behaviour experiments, 61 with females, 34 with males and 28 with juveniles. Spiders never exhibited a side bias (experiments 1 through 5, respectively: $p = 0.23$; $p = 0.83$; $p = 0.35$; $p = 0.54$; $p = 0.54$, Binomial test). When given a choice between abstract representations of their preferred prey and a realistic image of non-preferred prey (a house fly), *E. culicivora* chose the preferred prey significantly more often (experiments 1 and 2 respectively, $p < 0.001$; $p < 0.01$, Binomial test, Table 3). Spiders also chose a disarranged abstract representation of their preferred prey significantly more often than they chose a realistic image of non-preferred prey (experiment 3, $p < 0.05$, Binomial test, Table 3), or a disarranged non-prey item (experiment 5, $p < 0.05$, Binomial test, Table 3). However, spiders showed no preference when presented with an abstract representation of their preferred prey and a disarranged version of that same image (experiment 4, $p = 0.84$, Binomial test, Table 3).

Table 3: Results of two-choice predatory behaviour experiment. Pairs of stimuli used in the two-choice predatory behaviour experiments, number of pounce choices for each image, and results of Binomial tests. Note all stimulus sizes are equivalent, see Table 1.

Experiment	N	Image 1	Image 2	Chose Image 2	p
1	22			9%	<0.001
2	28			25%	<0.05
3	28			29%	<0.05
4	24			46%	0.84
5	21			19%	<0.05

Discussion

This study shows that for *E. culicivora*, discrimination and categorization can be achieved using only visual representations of the basic elements of its preferred prey. By using stick figure drawings of their preferred prey – *Anopheles* mosquitoes, we have created stimuli constructed only of key elements of their prey that have been found to be important for recognition (Nelson and Jackson 2006, Nelson and Jackson 2012). As hypothesised, we have shown that not only do these spiders view these stimuli as potential prey (by initiating predatory behaviour), but they also prefer these abstract images of prey to detailed images of alternative non-preferred prey. These results show that the various elements that have been found to be necessary for prey discrimination in previous studies (Nelson and Jackson 2006, Nelson and Jackson 2012) are also sufficient for recognition. This was the case regardless of whether or not the spiders had encountered their preferred prey before. Our controls have ruled out external cues, such as side preference, number of elements of the stimulus, and the relative contrast of the stimuli. Interestingly, the propensity to pounce was not affected by the different stimuli, and was seen in almost all cases where stalking was initiated. It would seem that the decision to pounce relies on other cues not singled out in this study, or, perhaps more likely, that pouncing is a follow-up behaviour akin to a 'fixed action pattern'.

Our confidence in these results is strengthened by the behaviour of the naïve juveniles in the single-choice predatory behaviour experiment. When hunting *Anopheles*, but no other type of prey, juveniles of *E. culicivora* perform an innate prey-specific predatory behaviour involving a detour to approach the prey from behind (Nelson et al. 2005). This detouring approach to the prey was evident in 57% of the trials involving a stimulus representing an *Anopheles* (stimuli 1-4; N = 31; stimulus 1 (detours/attacks): 7/11; stimulus 2: 4/9; stimulus 3: 6/10; stimulus 4: 1/1) with juveniles, but only once with the fly stimulus (stimulus 5; N = 8) and never with the circle stimulus (stimulus 6; N = 3). Despite these small sample sizes, it is apparent that they recognise the stick-figure stimuli specifically as *Anopheles* mosquitoes.

The low level categorisation of the abstract stimuli into prey and non-prey items is also seen in other invertebrates such as the praying mantis, where basic features of the stimuli, including size and speed, are the main cues (Prete et al. 2002, Prete et al. 2011). However, *E. culicivora*'s discriminations use much finer details of an image, such of the size and shape of mosquito antennae, when making decisions regarding preference (Nelson and Jackson 2006, Nelson and Jackson 2012), and thus require a considerably higher level of feature detection. The most notable instance of such discrimination in this study was the ability of the spiders to discriminate between the two disarranged stimuli in the single-choice predatory behaviour experiment, where the only difference between the stimuli were the relative angles between

the elements and yet one was categorised as prey, while the other was not. Nelson and Jackson (2006, 2012) have shown that the resting posture of a mosquito is an important cue for recognition. Our findings fine-tune those conclusions by suggesting that it is not the angle of the body compared to a surface or horizon, but rather the relative angles between the body elements that is crucial for recognition.

Discrimination of orientation has been shown in honeybees (*Apis mellifera*), which can distinguish different orientations even when these are produced through illusory contours (Van Hateren 1990) and without clear edge detection (Horridge 2000). Horridge (2009a,b) proposed that the generalization ability of the honeybee uses different parameters of an image to form local cues. These discrimination mechanisms were based on physical aspects of an image, but Avargues-Weber et al. (2011, 2012) demonstrated that honeybees are even capable of abstract concepts such as above-below and left-right. Unlike in the bee studies, unlearned stimuli and untrained animals were used, and show that *E. culicivora* is capable of discrimination using a significantly more complex abstract concept - angles between disconnected elements.

One way of achieving such discrimination ability is by storing the 'correct' orientation of the various elements and comparing each element to stored memory. However, the spiders occasionally pounced upon the stimulus while standing on the sides or the ceiling of the starting chamber (analogous to behaviour common in a natural setting, XJN pers. obs.), suggesting that orientation effects do not play a role in these decisions. While it is tempting to consider this type of object consistency in recognition to be superior to that seen in human recognition of faces (where face recognition is degraded significantly more than other objects when viewed upside-down (Yin 1969, McKone et al. 2007, Piepers and Robbins 2012, Richler et al. 2012), there is an inherent difference between the two - faces often have a prototypical orientation, while in the spider's natural three-dimensional environment prey is often viewed from different orientations.

An alternative mechanism of achieving the discrimination ability seen in this study is by 'calculating' the relative difference of the angles and comparing that to stored angles that represent prey. While discrimination of orientation has been well studied in vertebrates and invertebrates (Appelle 1972, Maske et al. 1986, Douglas and Hawryshyn 1990, Shashar and Cronin 1996, Srinivasan 2010), relative angle discrimination in non-human animals remains largely unstudied. In humans, however, this ability has been well studied (e.g., Marr and Nishihara 1978, Chen and Levi 1996, Kennedy et al. 2006) and there is some evidence for a neural mechanism that encodes angles in humans (Regan et al. 1996), as well as in macaques (Ito and Komatsu 2004) and cats (Shevelev 1998).

Our results demonstrate that *E. culicivora* not only categorises the simplified abstract stimuli as prey, but recognises them as its preferred prey, exhibiting higher level categorization or within-category discrimination. This was the case even for the disarranged version of the blood-fed *Anopheles*, a capability not dissimilar to that of humans with visual expertise when viewing fragmented images of cars or faces (Harel et al. 2011), although in this case the images were abstract and dispersed rather than fragmented. *E. culicivora* not showing any preference between the blood-fed *Anopheles* stimulus and its disarranged version was perhaps the most surprising finding of this study. While it is possible that *E. culicivora*'s response to the image of the disarranged *Anopheles* was due to its resemblance to some other unknown prey rather than *Anopheles*, this is unlikely as the dietary preferences of these spiders has been well studied (Jackson et al. 2005, Nelson et al. 2005, Nelson and Jackson 2006, Nelson and Jackson 2012). It should be noted that experiments using stimuli 4 and 7 were both run at a later date. While this too might have affected the results, this also seems unlikely, as the laboratory conditions were constant and the spiders were healthy. Another alternative explanation is that the specific arrangement of the elements of the disarranged *Anopheles* exploits a sensory bias in the *E. culicivora*'s visual pathways, while the altered version of this stimulus does not. Regrettably, we could not test the spider's responses to other alternative arrangements of these stimuli. Nonetheless, either through a sensory bias in the visual pathways, or by higher level visual analysis, the spiders evidently categorised both the blood-fed *Anopheles* stimulus and its disarranged version as their preferred prey. This suggests that they do not use a global, or holistic approach to recognition (Rock and Palmer 1990, Piepers and Robbins 2012), but rely instead on the analysis of specific elements at a local level to recognise an object (Kimchi 1992, Heinze et al. 1998, Förster and Higgins 2005). This type of analysis functions much like distributed feature extraction algorithms of object recognition in computer vision based upon the vertebrate visual cortex (Won et al. 2000, Lillywhite et al. 2013), in which low-level areas of the nervous system are delegated to recognizing different elements which are then fed to higher order centres (Sanes and Zipursky 2010). A closer look at how these spiders visually analyse what it is they are seeing will provide a deeper understanding of what specific features these spiders are looking for when they are looking for prey.

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Chapter 3

Biological relevance affects object recognition in jumping spiders

***Hypoblemum albovittatum* Mating (Photo: M. Walters)**

Dolev, Y. and Nelson, X. (2016). Biological relevance affects object recognition in jumping spiders. *New Zealand Journal of Zoology* **43**, 42-53**. *** Appendix 5

Abstract

In this chapter we investigated whether biological relevance affects the perceptual processes underlying prey classification in jumping spiders (Salticidae). Choice experiments with abstract and realistic representations of prey were used to test whether *Hypoblemum albovittatum*, a generalist predator, differs in how it classifies prey compared with *Evarcha culicivora*, which specialises in preying on blood-fed *Anopheles* mosquitoes. Unlike *E. culicivora*, *H. albovittatum* preferentially chose realistic over abstract representations of prey. Both species had similar decision times when choosing realistic images, which for *H. albovittatum* was similar to its decision time with abstract stimuli. In contrast, *E. culicivora* was significantly faster at making a decision with abstract images of *Anopheles*. These results suggest that *E. culicivora* uses key feature extraction methods when confronted with its preferred prey, but otherwise relies on holistic processing of an object, which appears to be the mechanism used by *H. albovittatum*.

Introduction

For any predator, a necessary prerequisite to prey selection is object recognition and categorisation, yet how animals achieve this fundamental task is surprisingly understudied. Visual object recognition is the ability to perceive the physical properties of an object (such as shape, colour and texture) followed by applying semantic attributes to the object (Enns 2004), such as the classification of the object as prey, predator or irrelevant. The diverse natural histories of visual predators have led to vastly different processes of classification of prey items. Some predators make rapid decisions and do minimal classifying of prey into particular types, instead relying on basic key features of an object as identifiers of prey. The use of key attributes is often used by amphibians (Barlow 1953; Lettvin et al. 1959; Heinze et al. 1998), mantises (Prete et al. 2011) and even birds (Bond 2007) to classify an object as prey. These elements include a specific size range, movement in a specific orientation, colour or pattern. In contrast, while little work has been done on visual discrimination abilities in predator–prey interactions, it is well known that many animals, including many invertebrates, are capable of learning complex visual discrimination tasks (Srinivasan 1994; Gierszewski et al. 2013; Fuss et al. 2014). As exemplified by the formation of search images, this ability can be used when hunting (Bond 2007). However, whether closely related animals categorise the same stimulus differently, or even possibly attribute different valence to it, has not been directly explored. Nonetheless, this might be expected when considering predators with different predatory behaviours and preferences.

Predators are commonly divided into generalists that consume a wide range of different types of prey or specialists which tend to consume specific types of prey more often than might be expected given their prevalence in the habitat. Predatory specialists therefore target specific types of prey, and express a preference for those particular prey types when given a choice. Perhaps one of the most interesting questions relating to prey preference is its evolution (Tauber et al. 1993; Pekár 2004; Pekár & Toft 2014) and the parallel evolution of the processes underlying object recognition and categorisation. This is because, crucially, the behavioural traits of prey preference and dietary specialisation rely on a predator's ability to distinguish between different types of prey. Consequently, a comparative approach between specialists and generalists within the same animal grouping is a powerful way to investigate both the perceptual processes underlying object classification, and the salience of different objects to specific animals.

Spiders are generally envisaged as generalists (Bristowe 1941; Wise 1993; Foelix 1996; Wise 2006), yet it is within this group, particularly among jumping spiders (Salticidae), that we find some of the most

extreme cases of prey specialisation known. These include spider-eating species (Jackson & Hallas 1986; Jackson 1992; Harland & Jackson 2000, 2006), ant-eating species (Edwards et al. 1974; Cutler 1980; Jackson & Li 2001; Jackson & Nelson 2012), and even a species (*Evarcha culicivora* Wesolowska and Jackson, 2003) that has a particular preference for blood-fed female mosquitoes in the genus *Anopheles* (Wesolowska & Jackson 2003; Jackson et al. 2005; Nelson & Jackson 2006). This East African spider is capable of using vision alone to discriminate between its preferred prey and similar-looking male *Anopheles*, female *Anopheles* that have not fed on blood, non-anopheline mosquitoes, as well as various similar-sized non-mosquito prey (Jackson et al. 2005; Nelson & Jackson 2006, 2012). In contrast, most salticids are generalists, showing no preference for specific prey when given the choice between different prey types. One such salticid is the New Zealand house hopper, *Hypoblemum albovittatum* (Keyserling, 1882). While little information is available on the natural diet of *H. albovittatum*, personal observations, as well as many years of experience in the laboratory (Tarsitano & Jackson 1992), led me to conclude that this is a generalist species.

Salticids are especially suited for investigation into visual processing and object categorisation because they are highly visual animals that respond readily to digital images on screens. Moreover, salticids are capable of discriminating minute details in a visual scene (Nelson 2010; Nelson & Jackson 2012). This is enabled by a pair of large forward-facing eyes (the Anterior Median or principal eyes), which are specialised for high resolution vision (spatial acuity), but within a very narrow (c. 2–5°) field of view (Land 1969a; Williams & McIntyre 1980; Land 1985; Blest et al. 1990). However, this narrow field of view is compensated for with complex retinal movements that scan up to c. 28° on either side of the body axis (Land 1969b). A tiered retina sits at the end of an elongated eye tube attached to the corneal lens, which is part of the exoskeleton. The eye tube is surrounded by six muscles, which enable horizontal, vertical and rotational movement even though the corneal lens is static (Land 1969b). In addition, salticids have three pairs of smaller, immobile lateral eyes with a combined visual field of c. 360°. These 'secondary eyes' function primarily as motion detectors (Land 1971, 1972; Zurek et al. 2010; Zurek & Nelson 2012).

We have previously shown that the mosquito-eating salticid *E. culicivora* not only categorises abstract stick figure representations of a mosquito as prey, but also recognises stick figure *Anopheles* mosquitoes as its preferred prey, even when the comprising elements of the *Anopheles* stick figure are disarranged and disconnected from each other (Chapter 2, Dolev & Nelson 2014). Our work on *E. culicivora* demonstrated that this species primarily uses feature extraction methods for recognising at least this kind of prey, without the need of holistic processing.

Here we test whether biological relevance affects the recognition and classification of abstract images of prey in two related predators. Specifically, we predicted that the predatory specialist salticid *E. culicivora* would differ in its ability to classify prey compared with *H. albovittatum*, a generalist salticid hunter, in accordance with the biological significance of the prey to the spider. We tested the prey choice behaviour of *H. albovittatum* in a multi-choice experiment using common prey items from the natural environment of this species, predicting that there would be no preference for any food item. We then used a two-choice test using abstract and realistic images to examine the responses of *H. albovittatum* to abstract images of *E. culicivora*'s preferred prey item—*Anopheles* mosquitoes. For this experiment we predicted that, unlike *E. culicivora*, *H. albovittatum* would preferentially choose realistic stimuli over abstract representations of prey. To test the effects of biological relevance, these results were compared with our previous results with *E. culicivora* (Chapter 2, Dolev & Nelson 2014).

Methods

(a) *General*

All testing was carried out between 0800 and 1400 h in a temperature-controlled laboratory set to 24 °C, with a photoperiod of 12L:12D (lights on at 0700 h). Spiders were housed individually in 1L plastic cages with a damp cotton wick for humidity. Spiders were fed to satiation once a week on Drosophila spp.; but before testing spiders were subjected to a 5–10 day fast. Test spiders were adult (body length, 4.5–5.5 mm) and juvenile (1.5–2.5 mm) *H. albovittatum*, a locally common and readily identifiable species. Females have a distinct abdominal pattern and a dark spot on the anterior dorsal part of their cephalothoraces, while males have dark legs and an orange band around their eyes (clypeus). All spiders were collected from houses and gardens around the University of Canterbury, and were kept in the lab for a minimum of 2 weeks before use. Gravid females were not tested.

All stimulus images used for tests (Figure 1) were created using Adobe Photoshop CS5. Figure 1a and 1c-f (from Crowe 2002) were rendered in black and white and were placed on a transparent background. Figure 1b, a circle approximately the size of a housefly, and Figure 1g, a photograph of an *Anopheles gambiae* Giles, 1902 mosquito in its typical resting posture, were also rendered in black and white with the background removed. Figure 1h was a stick figure representation of an *A. gambiae* in its typical resting posture (ensuring that the angles between all body parts were maintained) and Figure 1i was a scrambled disconnected version of Figure 1h, created so as to not alter the respective angles of any

of the elements of Figure 1h, while ensuring the elements were disconnected and, to humans, no longer resembling a mosquito.

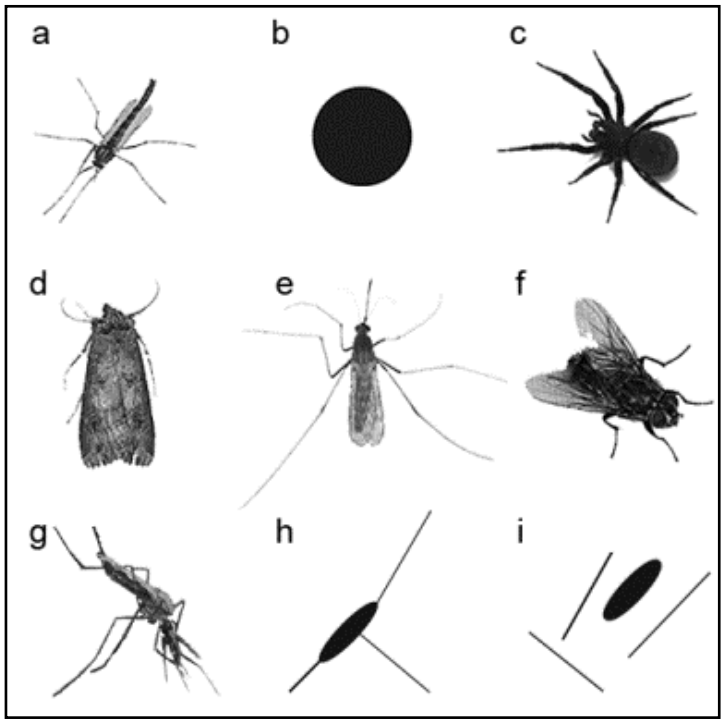

Figure 1: Stimuli used in the Multi-choice experiments
a. *Chironomus zealandicus* (midge);
b. circle;
c. *Badumna longinqua* (spider);
d. *Eudonia angustea* (moth);
e. *Culex pervigilans* (mosquito);
f. *Musca domestica* (fly);
g. *Anopheles gambiae* (mosquito);
h. *Anopheles gambiae* (stick figure);
i. disarranged *Anopheles gambiae* (stick figure).

(b) *Multi-choice experiment*

These tests took place in an arena where six stimuli (Figure 1a-f) were visible to the test spider. The arena was made from 5 mm PVC sheets and was created by placing six inclined (22°) ramps around a central hexagon (the 'starting platform') placed atop a PVC base (dimensions in Figure 2). Attached at the top end of each ramp was an electric stimulus mount built using a deconstructed analogue voltmeter, which was placed in front of a white background. The needles of the voltmeters were used to mount the different stimuli by gluing a thin tube to the back of each picture and then sliding the tubes over the needles. All voltmeters were connected to a control unit to trigger stimulus movement. Each trigger consisted of recurrent electric pulses, whose frequency, amplitude and duration could be controlled, causing the voltmeter needles to simultaneously 'jiggle' 15° to each side of the vertical for 2 s (at 5 Hz). These settings were designed to be most noticeable by the spiders, as determined by preliminary experiments.

The stimuli were printed life-size on standard photo paper and cut to size. Before each test, I the location of each stimulus within the arena was randomised and then the spider was placed on the starting platform at the centre of the arena (under a Petri dish) and was left to calm down for about 4 min. During this time, and throughout the test, the images were jiggled once every 30 s. Tests began when the spider was released.

We recorded every time the spiders noticed and stalked a stimulus. Noticing behaviour is characterised by the spider performing an optomotor response to face the stimulus with its anterior median eyes and subsequently staring continuously at the stimulus for several seconds. Stalking behaviour is characterised by the salticid slowly stepping towards the prey with its body lowered while visually fixated on the prey. Both are reliably identifiable behaviours commonly used in spider behaviour experiments (e.g. Nelson & Jackson 2012; Dolev & Nelson 2014). For this experiment, stalking of a stimulus was regarded as the spider making a choice. Sessions ended when the spider started stalking a stimulus, walked off the arena, or when 15 min had elapsed without the spider making a choice (the latter two were considered 'failed tests' and were used for analyses concerning attrition rate, see below).

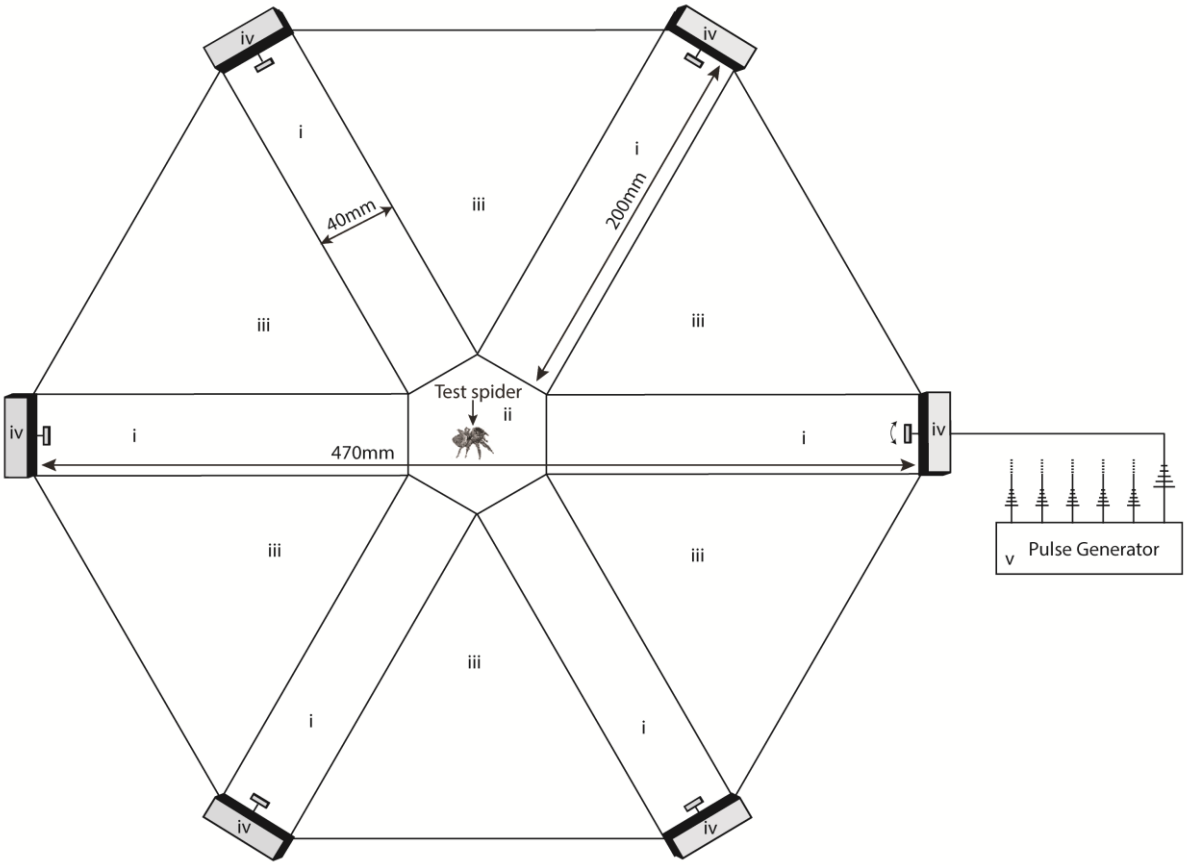

Figure 2: Test apparatus used for multi-choice experiment for *Hypoblemum albovittatum.*
i. angled PVC ramps (×6);
ii. starting platform;
iii. areas between ramps;
iv. holders on which stimuli were placed at the end of each ramp;
v. pulse generator connected to each stimulus holder.

(c) *Two-choice test*

For detailed methods see Chapter 2. Stimuli consisted of videos containing two stimuli (Figure 1f–i), which moved identically and simultaneously. Some of these stimuli were realistic depictions of potential prey (Figure 1f–g), while others were stick figure representations of prey (Figure 1h–i), to which *E. culicivora* responds in the same manner as realistic images (Chapter 2). Videos were back-projected onto a frosted glass screen through a lens placed 10 mm from the projector. Stimulus motion (two bouts of movement every 10 s) was at a speed of 16°/s and moved up and down at 8° visual angle from the starting position at which *H. albovittatum* were placed. These parameters were selected to maximise the attention of the spiders (Zurek et al. 2010).

Experiments were held within an apparatus containing a stainless steel ramp (15 mm wide × 150 mm long; angled up by 25°) in front of the screen. At a distance of 22 mm from the end of the ramp, a stainless steel 'starting box' (11 mm wide × 19 mm high × 22 mm deep; i.e. furthest point 44 mm from top end of ramp) was welded to the ramp complex. The box had a transparent Perspex 'door' wired to an external controller for remote opening. Before each session, which image was on the right and which was on the left was randomised. The spider was placed into the starting box and the door was closed for about 4 min as a calming period, after which, once the spider was away from the door of the starting box, the door was opened and tests began. Tests ended with the spider either pouncing on one of the two images on the screen or jumping/walking off the ramp. Failing these two conditions, tests were stopped after 15 min. Due to the short distance between the screen and the starting position on the apparatus, the spiders were able to pounce without stalking, so only pouncing behaviour was recorded.

(d) *Data Analysis*

All analyses were done using SPSS Statistics v.20, GraphPad Prism v.6 and R v.3.0.1. For the multi-choice experiments, $\chi 2$ tests were performed on the spiders' choices of stimuli as well as power analyses using a medium ($w = 0.3$; see Cohen 1988) effect size. To determine any compass orientation bias, the orientation of the ramp chosen in multi-choice tests was also analysed ($\chi 2$ tests). Spiders were divided into three groupings: female, male and juvenile (juvenile sex cannot be discerned). Kruskal-Wallis tests were used to investigate the effects of the sex or age on both prey choice and decision time (defined as the time elapsed between first noticing a stimulus and initiating stalking behaviour) in both the two-choice and the multi-choice experiments.

Binomial tests were used to analyse prey choice in the two-choice experiments, and Bonferroni-adjusted $\chi 2$ tests and Fisher's exact tests to analyse attrition rates. To analyse the decision time within and between each two-choice experiment, t-tests and, where appropriate, Mann-Whitney tests were used. These tests were also used to analyse data from our previous work on *E. culicivora* (Chapter 2), enabling me to directly compare the decision times and attrition rates for a specialist and for a generalist predator when viewing the same stimuli.

Results

In multi-choice tests, 41 (19 females, 15 males and seven juveniles) out of 123 sessions (33%) ended with the spiders stalking (choosing) an image. The spiders showed no significant preference for any of the six images (χ^2 = 1.585, df = 5, NS, w = 0.3, power = 0.98; Table 1) or the directionality of any of the ramps (ramp 1-6 respectively: *n*= 4, 8, 11, 9, 4, 5; χ^2 = 6.268, df = 5, NS). The sex or age of the spiders also had no significant effect on prey choice (*H* = 1.355, df = 2, NS; Table 1). Finally, there were no significant differences in the decision time between the different choices (*H* = 4.456, df = 5, NS; Table 1).

Table 1: Results from multi-choice prey tests for *Hypoblemum albovittatum.* F = female; M = male; J = juvenile; dt = decision time (s).

	Circle	**Fly**	**Midge**	**Mosquito**	**Moth**	**Spider**
Selected n (%)	4 (9.8)	8 (19.5)	7 (17.1)	7 (17.1)	8 (19.5)	7 (17.1)
Mean dt ± SEM	250 ± 114	489 ± 126	213 ± 39	244 ± 71	228 ± 101	188 ± 42
***n* for F; M; J**	3; 0; 1	4; 4; 0	2; 1; 3	3; 2; 2	4; 3; 1	3; 4; 0

In the two-choice experiments, *H. albovittatum* showed a significant preference for the detailed 'realistic' images over the abstract images (Table 2, experiments B and D). However, they showed no preference between the two realistic images used (house fly and mosquito) or the two abstract ones (stick figure mosquito and its disarranged version). There were significant differences in the attrition rates between the different choice tests (2 X 5 test, χ^2 = 19.7, df = 4, *P* < 0.001; Table 2), with the attrition rate in experiment A - which consisted of two abstract images - being significantly higher than in any other experiment (Fisher's exact test with Bonferroni adjustments, experiments A vs. B: *P* = 0.003; A vs. C: *P* = 0.002; A *vs*. D: *P* = 0.0007; A vs. E: *P* = 0.0015). There were no significant differences between any of the other pairwise comparisons. No differences were found when comparing the decision time between the experiments (*H* = 5.998, df = 4, NS; Table 2). Within each two-choice experiment, there were no significant differences in the decision time between the choices (experiments A-E [all NS], respectively: *t* = 1.046, df = 19; *t* = -1.223, df = 25, *P* = 0.233; *t* = -1.037, df = 28; *t* = 0.001, df = 27; *t* = 0.257, df = 21; Table 2).

Using unpublished data (Table 3) from the previous two-choice experiments on *E. culicivora* (Chapter 2, Dolev & Nelson 2014) we found significant differences in the decision times between the

choices within experiment G (U = 15.0, df = 2, P = 0.001), with the decision time for the abstract image representing a mosquito being shorter than for the realistic image of a fly (Figure. 1f-i). This was also the case for experiment H, although the sample size was too small for statistical analysis due to the extreme preference for one stimulus over the other (Table 3). There was no significant difference in the decision time between the two abstract images (t = 0.307, df = 22, NS; experiment F; Table 3).

Comparing decision times in the two-choice experiment of *H. albovittatum* and of *E. culicivora*, I found significant differences in the decision times when faced with abstract images (Figure 1h-i), with *E. culicivora*'s decision time being significantly shorter than that of *H. albovittatum* (Table 2, experiment C and Table 3, experiment G: U = 10.0, df = 2, P = 0.001; Table 2, experiment D and Table 3 experiment H: U = 36.5, df = 2, $P < 0.05$). No difference were found (t = 0.098, df = 29, NS) in decision times to choose a realistic image of a fly (Table 2, experiment C and Table 3, experiment G). When comparing experiments A and F (Tables 2 and 3), which have only abstract images (Figure 1h-i), the average decision time of *E. culicivora*, regardless of choice, was significantly shorter than that of *H. albovittatum* (U = 134.0, df = 2, $P <$ 0.01).

Comparing the attrition rates in the two-choice experiment of *H. albovittatum* and of *E. culicivora*, I found significant differences in the experiments that showed only abstract images (Fisher's exact test, Table 2, experiment A vs. Table 3, experiment F: $P < 0.001$), with the attrition rate of *E. culicivora* being significantly lower than that of *H. albovittatum.* No differences were found in the attrition rates between the salticid species in experiments that contained the realistic image of a fly (Fisher's exact test, Table 2, experiment C vs. Table 3, experiment G: P = 0.675; Table 2, experiment D vs. Table 3, experiment H: P = 0.811).

Table 2: Stimuli used and attack rate of *Hypoblemum albovittatum* in two-choice tests. dt = decision time (s). P values are of binomial tests. n = total spiders used, including those that made no choice.

Experiment	Image 1	Pounced on image 1 (mean dt ± SEM)	Image 2	Pounced on image 2 (mean dt ± SEM)	*P*	*n* (mean dt ± SEM)	Attrition (%)
A		12 (387 ± 40)		9 (323 ± 47)	0.66	63 (360 ± 31)	67
B		7 (398 ± 131)		20 (558 ± 63)	<0.05	42 (516 ± 58)	36
C		7 (466 ± 96)		23 (381 ± 35)	<0.05	47 (401 ± 35)	36
D		8 (332 ± 51)		21 (332 ± 80)	<0.05	43 (332 ± 42)	33
E		13 (473 ± 85)		10 (440 ± 96)	0.68	34 (458 ± 62)	32

Table 3: Stimuli used and attack rate of *Evarcha culicivora* in two-choice tests. dt = decision time (s). P values are of binomial tests. n = total spiders used, including those that made no choice.

Experiment	Image 1	Pounced on image 1 (mean dt ± SEM)	Image 2	Pounced on image 2 (mean dt ± SEM)	*P*	Total *n* (mean dt ± SEM)	Attrition (%)
F		13(187 ± 57)		11 (215 ± 74)	0.84	32 (200 ± 45)	31
G		20 (108 ± 34)		8 (375 ± 26)	<0.05	48 (185 ± 34)	42
H		20 (145 ± 26)		2 (496 ± 22)	<0.05	35 (177 ± 30)	37

Discussion

These data show how the biological relevance of a stimulus is manifested in the processes of object recognition, with the specialist predator *E. culicivora* quickly recognising abstract images as its preferred prey through feature extraction of key elements, while the generalist predator *H. albovittatum* appears to use no such short-cut for classification. Implicit representation is the use of key elements alone for creating a broad 'perceptual envelope' of images categorised as prey (Ewert 2004; Prete et al. 2011; Nelson & Jackson 2012). This process would be primarily beneficial for generalist predators making a broad category classification (i.e., 'prey'). We previously showed that *E. culicivora* recognises the abstract images of *Anopheles* mosquitoes specifically as its preferred prey (Chapter 2). This highly specific type of classification could not be the result of a broad-based implicit representation; rather, it seems that *E. culicivora* uses a narrow perceptual envelope to classify the stimulus, or 'implicitly identifies' the images specifically as *Anopheles*.

The results of the multi-choice experiment suggest that *H. albovittatum* is a generalist predator. With our relatively low sample sizes we can only rule out a strong preference to a particular prey item. However, for the purposes of this study we can reasonably consider *H. albovittatum* a generalist, as it initiated stalking behaviour roughly equally to each of the six stimuli used, suggesting that it categorised all images as potential prey items. Although a few individuals chose the circle (roughly half the number that chose the other stimuli), it is not entirely surprising that the spiders categorise the circle as a prey item. Many generalist predators, including some salticids, categorise stimuli as prey using only basic key features (Drees 1952; Barlow 1953; Lettvin et al. 1959; Heinze et al. 1998; Prete et al. 2011; Bartos 2013). What is somewhat surprising was the high attrition rate in this experiment, as salticids typically readily respond to stimuli in the lab (Harland et al. 1999). The most likely explanation is that, rather than stalk distant prey, *H. albovittatum* tends to pounce on nearby prey. This suggestion is strengthened by the lower attrition rates in the two-choice experiments, where stimuli were presented at a distance from which spiders could directly pounce on the target. Indeed, in the two-choice tests where a realistic image was presented to *H. albovittatum*, the attrition rates were no different than those of *E. culicivora* from our previous work (Chapter 2). Interestingly, when the spiders were presented only with abstract images, *H. albovittatum*'s attrition rate increased significantly while *E. culicivora*'s remained unchanged.

Similarly, the decision time prior to an attack on the realistic and abstract images differed between the specialist and the generalist. Given the nature of the two-choice experiment, decision time can be considered as a proxy for how long it takes the spiders to visually analyse an image. Unsurprisingly, both species took the same amount of time to analyse the image of the fly. However, *E. culicivora* analysed the

abstract images (including the scrambled version) significantly faster than *H. albovittatum*, and also significantly faster than they themselves analysed alternative stimuli.

Bednarski et al. (2012) show that the salticid *Phidippus audax* (Hentz, 1845) uses stimulus movement as the key element for categorisation as prey, thus adopting a very large perceptual envelope of this category (including a moving rectangle). In a similar set of studies, Bartos (2007, 2013) showed that the salticid *Yllenus arenarius* Menge, 1868 uses four key elements (stimulus length, movement type, congruent location of body parts and number of appendages) for the classification of a stimulus as prey. Furthermore, stimulus length and type of movement are used for classification into two prey categories with distinct escape risks requiring different attack strategies. These key elements create somewhat smaller perceptual envelopes than that of *P. audax*. Together with our data, these exemplify the differences in object categorisation and image analysis that seem to be the result of the evolution of predatory specialisation. One can imagine an evolutionary spectrum, starting with a generalist predator such as *P. audax*, which represents prey as anything that moves 'the right way'. As specialisation evolved, the use of more cues enables the creation of narrower perceptual envelopes and the application of specific prey-catching behaviours, accordingly. At the other end of the spectrum, we find a highly specialised predator, such as *E. culicivora*, with a strong preference for one specific prey which it 'implicitly identifies' and which triggers prey-specific predatory behaviour.

Hypoblemum albovittatum and *E. culicivora* appear to classify the same images as different things. *Hypoblemum albovittatum*, being a generalist predator, did not appear to categorise the abstract images as prey items, while *E. culicivora* not only categorised them as prey, but identified them as preferred prey. *Evarcha culicivora*'s ability to recognise the abstract scrambled mosquito relies on feature abstraction (Chapter 2) and here we show that this recognition and categorisation process is performed more rapidly for abstract stimuli than for realistic stimuli. *Evarcha culicivora* also processed abstract images more rapidly than the generalist salticid. This suggests that the feature extraction processing is a benefit that coevolved with predatory specialisation in *E. culicivora*. The processing of the realistic images used in these tests took longer. It is therefore reasonable to assume that, compared with the quick low-level feature extraction characteristic of preferred prey recognition, analysing images that lack these features might require higher-level holistic processing.

This work suggests that, compared with generalists, specialists may use streamlined categorisation processes that facilitate the rapid identification of stimuli relevant to their specific life histories. Indeed, one can envision that it is the very strength of the implicit representation strategy (basing prey recognition

or representation on 'algorithms', rather than underlying neural components) that has paved the way for the evolution of predatory specialisation, either by narrowing the subset of objects that elicit appetitive behaviours, or by having some of these objects elicit a stronger appetitive behaviour.

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Chapter 4

Retinal image scanning and active vision in jumping spiders

Adult male P. fimbriata *prior to being placed in front of the eye-tracker* (Photo: Y. Dolev)

Abstract

An important step in visual perception is the acquisition of information and details of the visual scene. To achieve this, many animals perform a set of retinal movements known as a visual search pattern. By analysing these search patterns we can get a glimpse into the way animals view the world. In this chapter, using an eye-tracker designed specifically for this task, we investigated the visual search patterns of four salticid species using a set of simple stimuli – a bar, a circle, two adjacent circles and two abstract face-on images of a salticid with and without legs. The exceptional eyesight of salticids is the result of multiple camera-type eyes. The two large forward-facing eyes - the anterior medial or 'principal' eyes - have a narrow field of view and movable retinae with high spatial acuity. Viewing these retinae through an eye-tracker, we found that rather than sequentially scanning the entire visual scene, salticids follow the edges of the stimuli with either the fovea at the centre of the retina or the retinal edges. Moreover, scanning movements were more active when the stimuli resembled an anterior view of a jumping spider than any of the other stimuli. These results suggest that the scanning movements are part of a closed-circuit system which is strongly affected by efferent innervation, shedding new light on the incredible capabilities of the miniature nervous system of these animals.

Introduction

How visual animals recognise scenes and the objects within them has fascinated researchers for decades (see Kowler 2011, Land and Nilsson 2012), and, for animals of vastly different taxa, this process begins with the movements of the eyes. Walls (1962) suggested that eye movements evolved to keep an image fixed on the retina, thus preventing the blur that occurs at angular velocities that exceed one receptor acceptance angle per photoreceptor integration time (Srinivasan and Bernard 1975; Land 1999). However, stabilisation alone is insufficient for a moving animal, which must occasionally re-centre its gaze through both long and short saccades (Land 1999). In addition, as the spatial acuity provided by photoreceptors of varying sizes varies across the retinal area (Land and Nilsson 2012), organisms need to make eye movements to direct the high-resolution region of the eye (often referred to as the fovea) toward objects of interest (Walls 1962). On the assumption that the background is stationary, eye movements facilitate the detection of motion of objects in the foreground (Land 1999). Together, through the pattern of stable fixations and fast saccades that shift gaze direction, a visual search pattern arises (Land 1999).

Visual search is constituted by a set of complex behaviours that encompass many aspects central to visual and cognitive function and can provide clues about how brains coordinate a variety of functions (Eckstein 2011). Consequently, visual search has been used as a framework to study many aspects of cognitive and visual function (Eckstein 2011), particularly in humans and other primates. A prime example is facial recognition work using gaze-tracking. Early studies tracking where humans 'look' (Yarbus 1969, Ellis et al. 1979, Young et al. 1985) found that certain areas of a face are more informative than others, with most fixations landing in the area around the eyes, nose and mouth (Luria and Strauss 1978), while the outline of a complex stationary image has little to no effect of the character of the eye movements (Yarbus 1969). These initial works formed the basis of recent research on face recognition suggesting that eye movements, typically considered a subconscious process, have functional roles and reflect not only the underlying cognitive processes (Henderson et al. 2005, Hsiao and Cottrell 2008), but also the emotional state of the subject (Schurgin et al. 2014).

Despite the successful application of gaze-tracking paradigms for primate visual research, attempts to develop similar methods for other animals are limited and typically on birds (Pietrewicz and Kamil 1979, Bond 1983, Langley et al. 1996, Dukas and Kamil 2001, Harmening et al. 2011, Schwarz et al. 2013, Sridharan et al. 2013). Gaze-tracking results from birds has further illustrated the strong connection between ecology and cognitive functions, such as attention and expectation, on the processes of visual search. For example, pigeons exhibit 'matching selection', which is a tendency to over select (i.e. gaze upon) the more common grain; furthermore, stimulus detectability is enhanced by recent experience

(Bond 1983). In accordance with search image formation (Krebs 1973, Curio 1976) and apostatic selection, where a predator overlooks rare prey types while consuming an excess of abundant ones (Bond and Kamil 1998), these findings beautufully dovetail to provide depth to our understanding of the evolution of colour polymorphism and crypsis (Bond 2007).

In one of the few arthropod examples whose visual search patterns have been investigated, jumping spiders (Salticidae) differ significantly from that of most other studied animals, which might be expected given the unique structure of their visual system. The salticid visual system is comprised of four pairs of simple (camera-type) eyes: one large pair of forward-facing eyes (anterior median, AM) and three smaller pairs of eyes: the forward-facing anterior lateral (AL) eyes, the lateral-facing posterior median (PM; reduced in most species), and the rear-facing posterior lateral (PL) eyes (Land 1985). These AL, PM, PL eyes are collectively known as 'secondary eyes' and possess wide fields of view, which jointly encompass c. 360^{o}, with considerable binocular overlap in the frontal fields of view of the AL eyes (Land 1971, Land 1972, Zurek et al. 2010, Zurek and Nelson 2012). These eyes have fixed lenses and a fixed, sometimes foveated, retina which conveys some shape detection, but their function appears to be primarily as motion detectors (Forster 1979, O'Carroll 1989, Zurek et al. 2010, Zurek and Nelson 2012). Movement in the field of view of the secondary eyes causes a quick optomotor swivel by the spider which results in the target being placed within the field of view of the AM, or 'principle' eyes. The AM eyes have narrow, boomerang-shaped retinae, subtending about 20° vertically by 1° horizontally in the central region, which is roughly six receptor rows wide (Land 1972). These eyes provide outstanding spatial acuity (as low as 0.04°) over a narrow field of view of 3-5° (Land 1969a,b, Williams and McIntyre 1980, Blest et al. 1990).

Rather than being comprised of saccades and fixations, the visual search patterns of salticids are a continuous scan of the outside world with their AM eyes, unless they stop at a midpoint resting position, resulting in a continually changing retinal image (Land 1969a, Land 1999). Land (1969a) discovered that the AM retinae, which lie at the end of two long innervated 'eye tubes', are capable of vertical, horizontal and even rotational (torsional) movement. These degrees of freedom enable complex movements that can scan up to c. 28° from the central body axis - not only compensating for the narrow field of view of the AM eyes, but appearing to be responsible for the detailed analysis of the spatial features of a visual scene. Eye-movements are usually highly conjugated, with movements made by one retina being paralleled by the movements of the other, but the retinae can also move independently (Land 1969a,b). In his seminal work, Land (1969a) distinguished four distinct kinds of movements: (i) spontaneous activity, (ii) tracking motion, (iii) saccades and (iv) scanning movements. Spontaneous activity consists of bouts of periodic side-to-side movements of the retinae, which change in frequency, amplitude and regularity, at speeds varying between $2°s^{-1}$ to $100°s^{-1}$ for short bursts. Tracking motions are smooth movements of the retinae while

following a moving target over angles of at least 25° both vertically or horizontally. Saccadic motions are analogous to human saccadic eye-movements, whose goal is to bring a target seen in the periphery (in the case of salticids, by the secondary eyes) on to the fovea. Saccades are very rapid movements of at least 15° within 100 ms and are often accompanied by a rapid optomotor rotation by the spider. Following a saccade, the retinae then start either scanning motions or return slowly to their resting position (Land 1969a). When presented with a novel target, Land (1969a) states that the eyes scan it in a stereotyped way, moving from side to side at speeds between 3 and 10°s^{-1}, while rotating through ± 25°.

Salticids overcome motion blur associated with fast image speeds by keeping tracking and scanning velocity just slow enough to stay within the limit of blurring. Additionally, the segregation into eyes with different properties and specialities overcomes problems associated with continuous retinal image motion, with the stationary secondary eyes dedicated to motion detection (Land 1999). However, although the secondary eyes are primarily thought of as motion detectors, the fact that the fields of view of the AL eyes completely overlap those of the AM eyes, coupled with their sometimes being foveated and having some shape detection capabilities (Land 1971, Land 1972, Forster 1979, O'Carroll 1989, Zurek et al. 2010, Zurek and Nelson 2012), suggests that the one of the roles of the AM eye movements is the accusition of aditional detail, perhaps even guided by the AL eyes. This type of visualy-guided eye (or camera) motion with the goal of accuiring additional detail is known as active vision, a term originating from the field of computer vision (Aloimonos et al. 1988) which has since been adopted by psycologists and biologists (Lehrer and Srinivasan 1994, Dawkins and Woodington 2000).

Land (1969a) recognised that the scanning motions of the AM eye retinae are concerned with feature extraction. Based on the work of Oscar Drees (1952), Land (1969a) suggested that the features being looked for are the legs of conspecifics, citing the rotational motions as evidence that they are looking for appendages at specific angles and using their elongated retinas as leg detectors. However, more recent work has showed that salticids correctly identify very different and highly specific stimuli. Harland & Jackson (2001) showed that *Portia fimbriata* distinguishes between jumping spiders (its preferred prey) and other spiders as well as insects. Legs alone would be insufficient for distinguishing between many spider species, and certainly between conspecifics and salticid prey. Further, *P. fimbriata* is affected by the presence-versus-absence of AM eyes, and by the position, size and shape of these eyes when identifying stimuli as salticids (Harland and Jackson 2002). Similarly, Nelson & Jackson (2006a) showed that *Myrmarachne assimilis*, an ant-like salticid which is a Batesian mimic of *Oecophylla smaragdina*, the Asian weaver ant, has the ability to differentiate, even when limited to sight alone, between models, conspecific individuals and prey. Later, using another ant-like salticid, *M. bakeri,* Nelson (2010) found that chelicerae, legs I and body shape, irrespective of movement, are important cues to distinguish conspecifics from ants.

This ability requires a high level of visual discrimination, one which its predators, including other salticids, fail to pick up on (Nelson 2012). Another intriguing example of sophisticated visual discrimination can be found in *Evarcha culicivora*, an East African salticid that preferentially preys upon blood-fed female *Anopheles* mosquitoes (Wesolowska and Jackson 2003). Unsurprisingly, its highly specific dietary preference is accompanied by impressive discrimination abilities which includes discriminating details as fine as the resting posture of its prey (Nelson and Jackson 2006b), the shape of the mosquito's abdomen, and even the structure of the antenna (Nelson and Jackson 2012). Perhaps even more astonishing is this ability to discriminate using conceptual, rather than visual, elements, such as the 'imagined' angle between two non-touching elements of a stimulus, while maintaining image persistence (Dolev and Nelson 2014).

Here we use an eye-tracker which is based on the ophthalmoscope design originally used by Land (1969a,b) to track the retinal movements of the AM eyes when viewing a series of simple stationary stimuli. The aim of this initial study was to asses and decipher the scanning patterns associated with feature extraction and object recognition. In addition to the ability to record video of the moving retinae, a key difference in the optics of this eye-tracker, when compared to the ophthalmoscope used by Land (1969a,b), is the wider field of view it provides. This enabled us to follow the movement of the eyes as the spiders scan their visual field. Here, using the same set of visual stimuli which differed in terms of their biological salience and complexity, four different species were tested: two specialist predators and two generalist predators. Using a comparative approach, this work serves as a first step in revealing the characteristics of retinal scanning performed by salticids with different ecological niches.

Methods

The four species used were *Portia africana, Sandalodes bipenicillatus*, *Servaea incana* and *Marpissa marina*. These species were similar in adult body size (c. 8-10 mm), but differed in the size of their retinae, with *P. africana* having much smaller retinae than the other species (see results). *Portia africana* is known for its specialised hunting behaviour and preference for spiders as prey (Jackson and Hallas 1986; Jackson & Nelson 2011), while *Sandalodes bipenicillatus* has a strong preference for ants (Zabka 2000; Nelson and Card 2015). *Servaea incana* is a generalist predator hunting insects on and under the bark of trees (Richardson and Gunter 2012, McGinley et al. 2016) and *Marpissa marina* lives on rocky shores and is also a generalist predator (Vink 2015). Spiders were housed and maintained as specified in Chapter 2. For each species, six unmated adult female spiders that were starved for 5-7 days prior to testing were used as test subjects. All tests were carried out between 08:00 and 14:00.

The eye-tracker (see Appendix 2) is specially designed to enable the recording of salticid retinal activity in near-infrared light while simultaneously displaying digital stimuli (in visible light). The eye-tracker used in this study (Appendix 2, Figure S2, S3) is a slightly modified version of the eye-tracker described in Canavesi et al. (2011). Stimuli were back-projected onto a white screen through a lens placed 10 mm from a projector (AAXA M2 micro projector), with the screen and lens being held in place using a 3D printed 'spider theatre' attached to the eye-tracker (Appendix 2, Figure S3). The stimuli (Figure 1) were simple black and white images created in Adobe Photoshop CS6. Images i to vi, respectively, were: a horizontal bar, a square, a circle, two circles side by side, four circles (two large, two small) arraged in an anterior view of the salticid eye configuration, and a stick figure image of the anterior aspect of a salticid (i.e., stimulus v coupled with the first pair of legs).

Test spiders were tethered in place using dental microapplicators dabbed in bees wax and then attached to the spiders' cephalothorax. The spider, attached to the microapplicator, was then placed in a micromanipulator, allowing fine positioning of the spider in front of the eye-tracker (see Appendix 2, Figures S3, S6 and S7). A polystyrene ball (diameter of 10-15 mm, depending on the size of the spider) was placed under the spider for it to freely walk on. While the balls were heavier than the spiders, they were light enough for the spiders to hold onto and turn easily, and no signs of fatigue were evident (see Zurek et al. 2010).

Once a clear image of the spider retinae was obtained, the test session began by presenting the stimulus. Each session consisted of a 5 min rest period followed by presenting the six different stimuli in random order. Each image was presented for 2 min with a 2 min inter-stimulus-interval between stimulus presentations. Sessions where the test spider showed no eye movements were discarded from analysis.

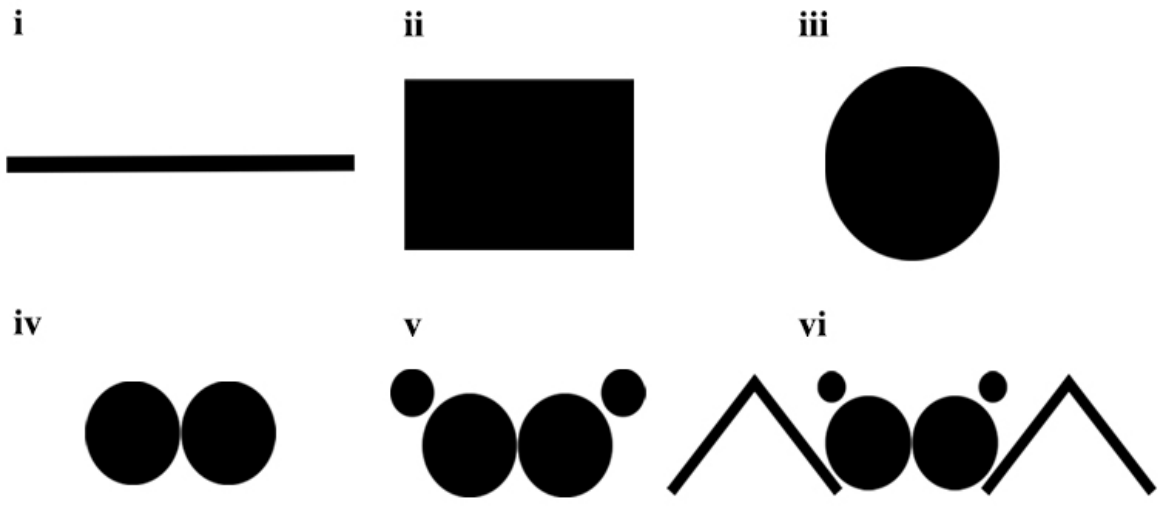

Figure 1: The six different stimuli used in this study. Relative scale of the different stimuli to each other is correct. i to vi respectively: a horizontal bar, a square, a circle, two circles side by side, four circles (two large, two small) arraged in an anterior view of the salticid eye configuration, and a stick figure image of the anterior aspect of a salticid.

The resulting 120 s video for each stimulus was split into its 3000 individual frames which were contrast enhanced (see Appendix 2). For each frame, the x,y coordinate of the fovea of each retina was recorded, using either machine learning object recognition software developed in Matlab when the image quality was sufficient, or using the MTrackJ plugin in ImageJ.

As we were only interested in the scanning motions, x,y coordinates where the retina (for each retina separately) did not move more than three pixels for five frames (i.e., identical frames or drift) were identified and removed. The resulting datasets, which were aggregated for each species and each stimulus, therefore represent only active retinal motion and do not depict the retinae at rest or unmoving.

Prior to analysis of the retinal movements, the average area, circumference and arm length (in pixels) of the eye-tracker image of the retina were calculated for each species, using a sample of fifteen images from each spider. Following that, the distances (measured in pixels) between the fovea of each retina and the nearest point on the outline of the stimulus (whose position was determined as '0') were measured for each frame, with negative values being assigned to foveal x,y locations within the stimulus, and positive values being assigned to x,y locations outside of the stimulus (see figure 3i). Due to differences in retinal size (see Table 1, Figure 2) these distance data were normalised by dividing the distance by the average retinal 'arm' length (i.e., half the size of the boomerang-shaped retina) for that species (n = 15 per species). The reason for using this method for normalisation was that the resulting scale indicates which part of the spiders' retina was over the stimulus, with point 0 referring to the fovea, and 1

and -1 referring to a distance equivalent to one retinal arm from the stimulus edge. Scanning heatmaps (equivalent to gaze heatmaps in mammals) were created for each species and for each stimulus (using a window size of 20 pixels). For each stimulus, the heatmaps were normalised across species so that their scale was the same. Separately, we also produced heatmaps normalised across stimulus for each species.

Distance data were compared between the different stimuli within each species using Friedman's tests and pairwise comparisons between stimuli were analysed using Wilcoxon signed-rank tests with Bonferroni adjustments. Additionally, distance data were compared between the different species within each stimulus. These comparisons were done using Kruskal-Wallis tests and, for pairwise comparisons, Kolmogorov–Smirnov tests with Bonferroni adjustments. As a separate analysis, Kruskal-Wallis tests were used for pooled data across all stimuli to investigate the total number of frames in which the retinae moved across species (i.e., retinal activity as a function of species). Finally, a Mann-Whitney U test comparing the pooled data for the *a priori* defined groupings of predatory specialists (*P. africana* and *S. bipenicillatus*) and generalists (*M. marina* and *S. incana*) was used to determine retinal activity as a function of predatory specialisation. All analyses were done using Prism v6 and SPSS Statistics V20.

Results

The average sizes of the retinae of the different species are summarised in Table 1. As the size in pixels is of little relevance, the sizes are given as a percentage of the average size of the largest retina, that of *S. bipenillatus*. It is important to note that these sizes are of the retina as they appear on the screen through the eye-tracker and thus serve merely as an indicator to the actual size of the spiders' retinae because a number of different physiological properties can affect how the retinae appear on the screen. For example, the actual size of the retina, the reflective properties of the retinal and surrounding tissue, or the distance of the retina from the corneal lens (i.e., axial length, see Chapter 6). As these data were not collected, the actual retinal sizes cannot be addressed in this study.

Table 1: Retinal sizes and standard deviation (SD), expressed as percentages, of the different species used in this study, with the retina of *S. bipenicillatus* set as 100%. N = 15. Circumference: average total perimeter of both arms. Arm length: average of both the top and bottom arms.

Species	Area; SD	Circumference; SD	Arm length; SD
S. bipenicillatus	100; 17.9	100; 8	100; 7.5
S. incana	92.5; 10.8	98.4; 7.7	98.5; 5.4
M. marina	92.8; 12.2	104.9; 6.6	99.3; 4.5
P. africana	20.7; 2.9	54.4; 4.3	56.6; 5.1

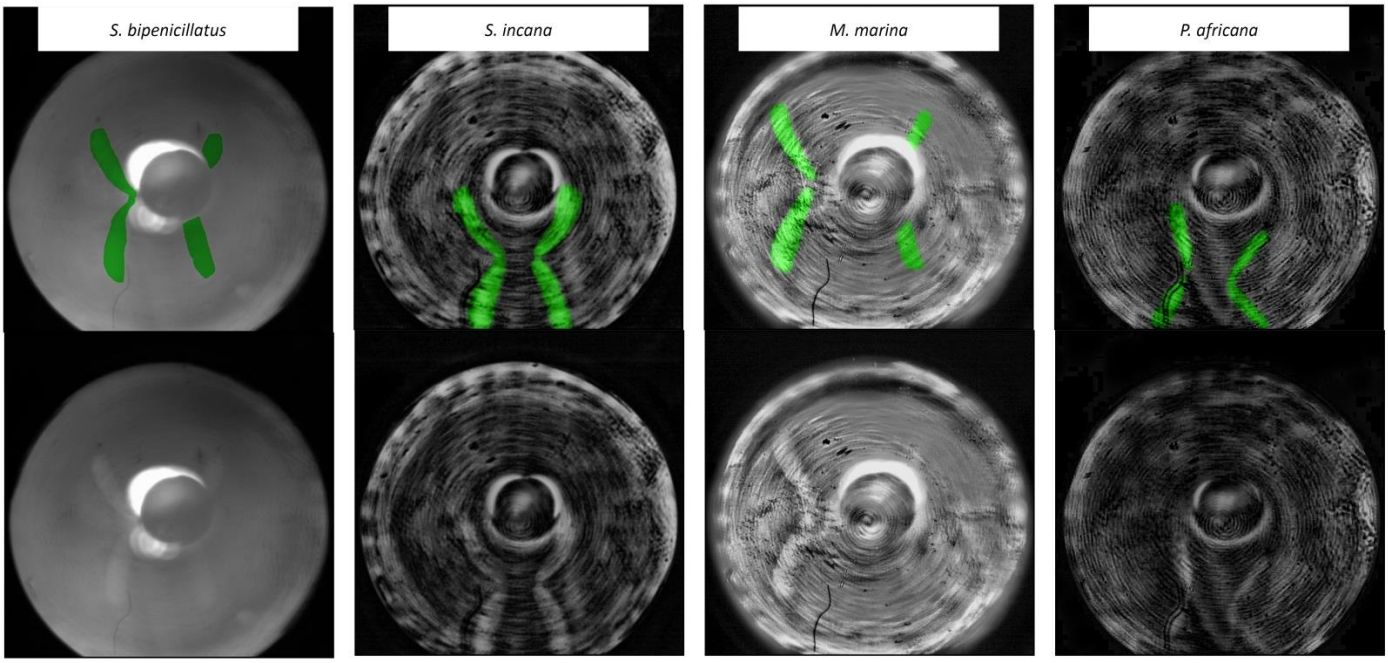

Figure 2: Images of the retinae for the four species used in this study as seen through the eye-tracker (top: false colour applied to the area of the retina; bottom: original images). The different backgrounds are due to different image enhancement protocols that were applied as needed.

(a) *Scanning frame analysis*

Comparing the combined (i.e., regardless of stimuli) total number of frames in which there was retinal movement ('scanning frames') between the grouped data of the specialists and generalists, a significant difference was found ($U = 1441$, $p < 0.05$); however Bonferroni-adjusted pairwise comparisons between the different stimuli showed no significant differences. Similarly, when comparing the number of scanning frames between the four species separately (again, regardless of stimuli) significant differences were found ($\chi^2 = 9.791$, $df = 3$, $p < 0.05$), although Bonferroni-adjusted pairwise comparisons showed that there were significant differences only between *S. incana* and *P. africana* (Table 2).

Table 2: Mann-Whitney pairwise comparisons of scanning frame numbers between species. Significant differences indicated in bold.

	M. marina	***S. incana***	***S. bipenicillatus***
S. incana	U = 327; p = 0.078	-	-
S. bipenicillatus	U = 410; p = 0.895	U = 373; p = 0.063	-
P. africana	U = 351; p = 0.16	U = 307**; p < 0.005**	U = 434.5; p = 0.301

Comparing the number of scanning frames between the four species for each stimulus separately showed that only for the square stimulus were there significant differences ($\chi^2 = 7.956$, $df = 3$, $p < 0.05$),

although Bonferroni adjusted pairwise comparisons revealed no significant differences. Finally, in comparing the number of scanning frames between the different stimuli for each species separately, no significant differences were found.

(b) *Within species comparisons of scanning distance*

For the normalised distribution of scanning distances from each of different stimuli for each of the four species significant differences were found (*M. marina:* $\chi^2 = 4883.27$; *P. africana*: $\chi^2 = 4829.77$; *S. incana*: $\chi^2 = 4884.46$; *S. bipenicillatus*: $\chi^2 = 7468.90$; in all cases df =5, $p < 0.0001$). With three exceptions (*S. incana*: Square vs. Eyes, Z = -0.785, p = 0.432; *M. marina*: Square vs. Spider, Z = -2.081, p = 0.005; *S. incana*: Circle vs. Eyes, Z = -2.436, p = 0.015 – the latter two were not significant after Bonferroni adjustment), Bonferroni-adjusted pairwise comparisons between the different pairs of stimuli (4 x 15 = 60 comparisons) yielded highly significant differences, with these being $p < 0.0001$ in all cases.

(c) *Within stimulus comparisons of scanning distance*

For each of the six stimuli, comparisons of the distribution of distances between the four species also showed significant differences (in all cases df =3, $p < 0.0001$; Bar: $\chi^2 = 1335.88$; Square: $\chi^2 = 2590.79$; Circle: $\chi^2 = 4354.28$; 2 Circles: $\chi^2 = 2692.78$; Eyes: $\chi^2 = 5597.73$; Salticid: $\chi^2 = 553.16$; Figures 3-8, respectively). Bonferroni-adjusted pairwise comparisons between the different species were also significant ($p < 0.001$) in all 6 x 6 cases.

Although these statistics suggest that there are no trends, the histograms suggest that there are some common features, and it may be that larger sample sizes are needed to accurately assess the retinal scanning of these salticids. One standout feature is that in all but the circle stimulus, the 'hottest' values in the heatmaps are among the two specialist species (*P. africana* and *S. bipenicillatus*), suggesting a more focused approach to scanning. Below is a descriptive analysis of the results, segregated by stimulus.

(d) *Bar*

The distance histograms for the bar stimulus (Figure 3, a-d) are all bell shaped distributions with very low levels of negative values, most likely the result of the narrowness of the stimulus. For *P. africana* and *S. incana* the peaks are around one retinal arm distance from the stimulus edge, which is about twice that of *M. marina* and *S. bipenicillatus*. The corresponding heatmaps show that the hottest regions for all four species are along the edge of some part of the stimulus, with other hot spots above or below the stimulus, possibly suggesting the use of the edge of retinal arm for stimulus edge detection. Interestingly, none of the species tracked the entire edge of the stimulus, but rather focused on a small portion of it.

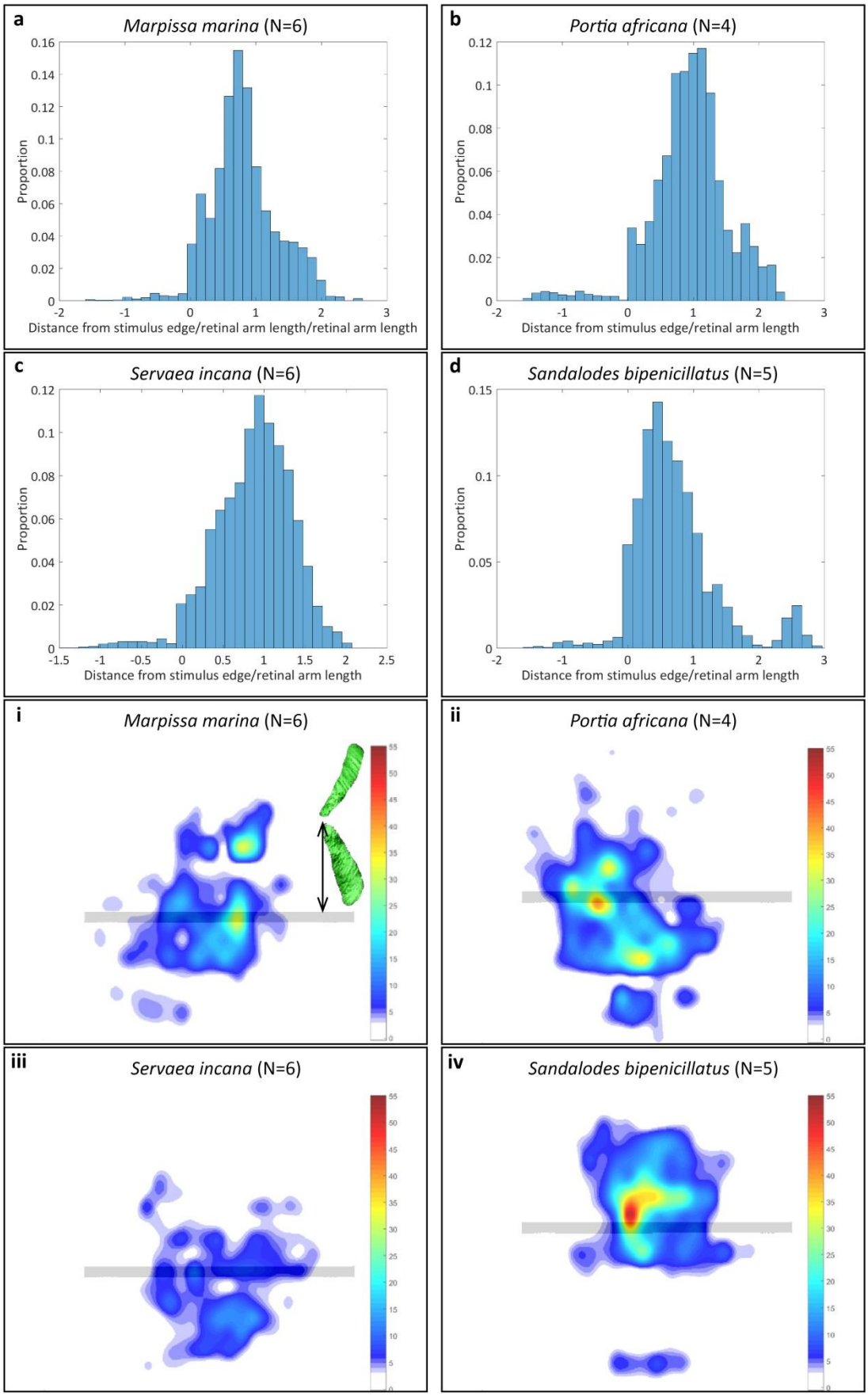

Figure 3: Distance histograms and corresponding heatmaps for the Bar stimulus for all four species. Heatmap values have been normalised to the highest value, in this case for *S. bipenicillatus* (image iv). The retina (not to scale) and arrow in image i depict an example of the distance measurements used for the histograms.

(e) *Square*

The histogram distributions for all species (Figure 4, a-d) are roughly bimodal, with a pit around point zero, signifying little attention of the fovea around the edge of the stimulus itself. For *M. marina* the highest peak is Just below zero, or just inside the stimulus (Figure 4a), which corresponds to its heatmap (Figure 4i), while the second peak is at a distance of around one retinal arm, although the corresponding heatmap doesn't suggest the use of retinal edges for stimulus edge detection. For *P. africana*, *S. incana* and *S. bipenicillatus*, much like *M. marina*, the dual peaks fall just inside the stimulus and between 0.6 - 1.5 retinal arm lengths away from the stimulus. However, unlike *M. marina*, the corresponding heatmaps shows that the different species focused mainly on the inner bottom right edge of the square (the first peak in the histogram), and below the square, suggesting the use of part of the retinal arm for edge detection.

(f) *Circle*

The histograms for the circle stimulus (Figure 5, a-d) are bimodal for *M. marina, S. incana* and *S. bipenicillatus* and somewhat trimodal in *P. africana*. Much like the square, the circle stimulus also resulted in strong negative values, possibly due to the large area of the stimulus. The highest peak for both *S. incana* and *S. bipenicillatus* is around one retinal arm length with a strong dip around the edge itself. Taken together with the corresponding heatmaps, this suggests the use of retinal edges for stimulus edge detection. In contract, the highest peak for *P. africana* is around 0-0.25, showing strong foveal focus on the edges of the stimulus, with two other peaks around half and one retinal length, respectively. For *M. marina* the majority of focus is on the inside of the stimulus (c. -0.4 retinal arm lengths), with the second peak c. two retinal arm lengths away.

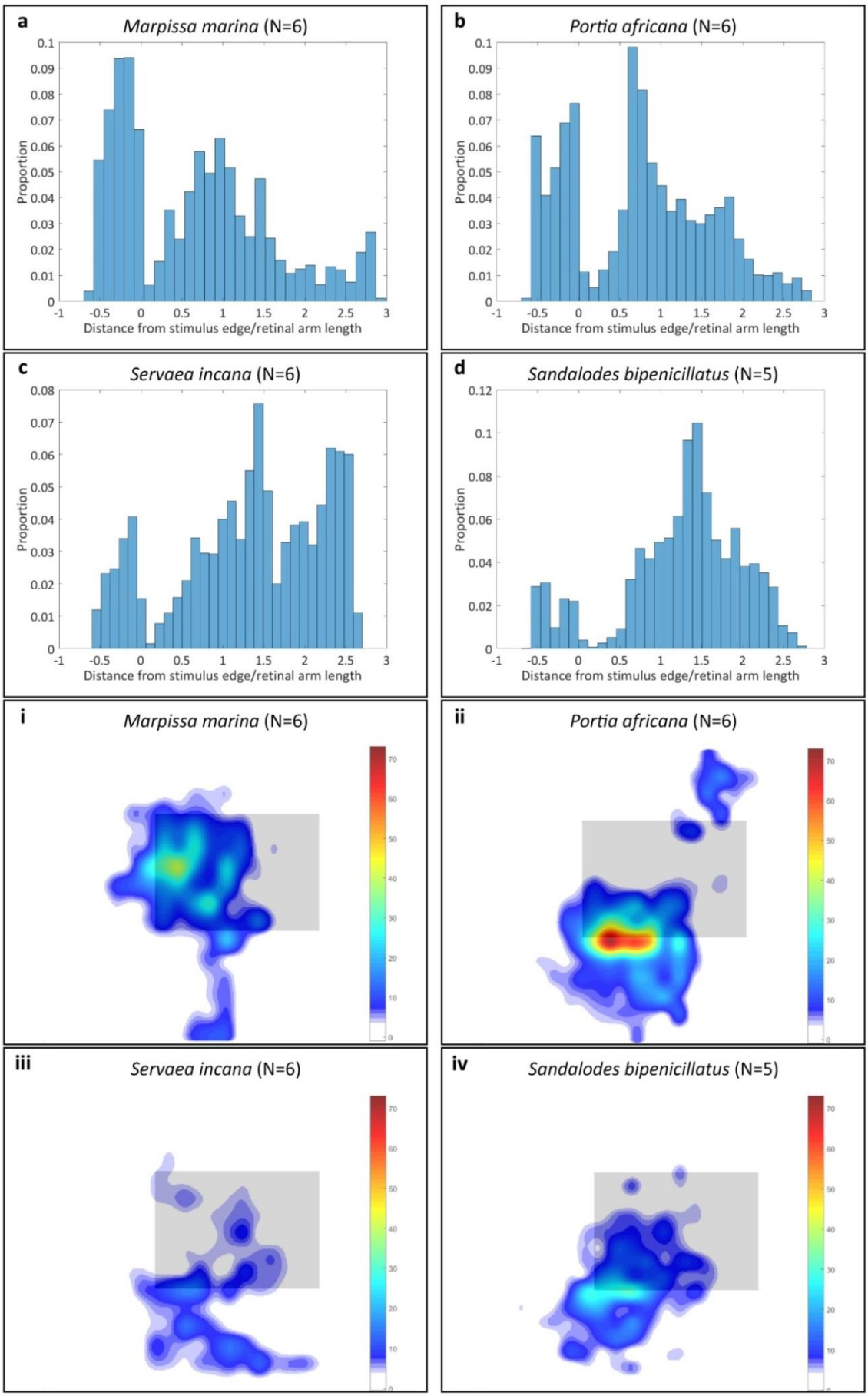

Figure 4: Distance histograms and corresponding heatmaps for the Square stimulus for all four species. Heatmap values have been normalised to the highest value, in this case for *P. africana* (image ii).

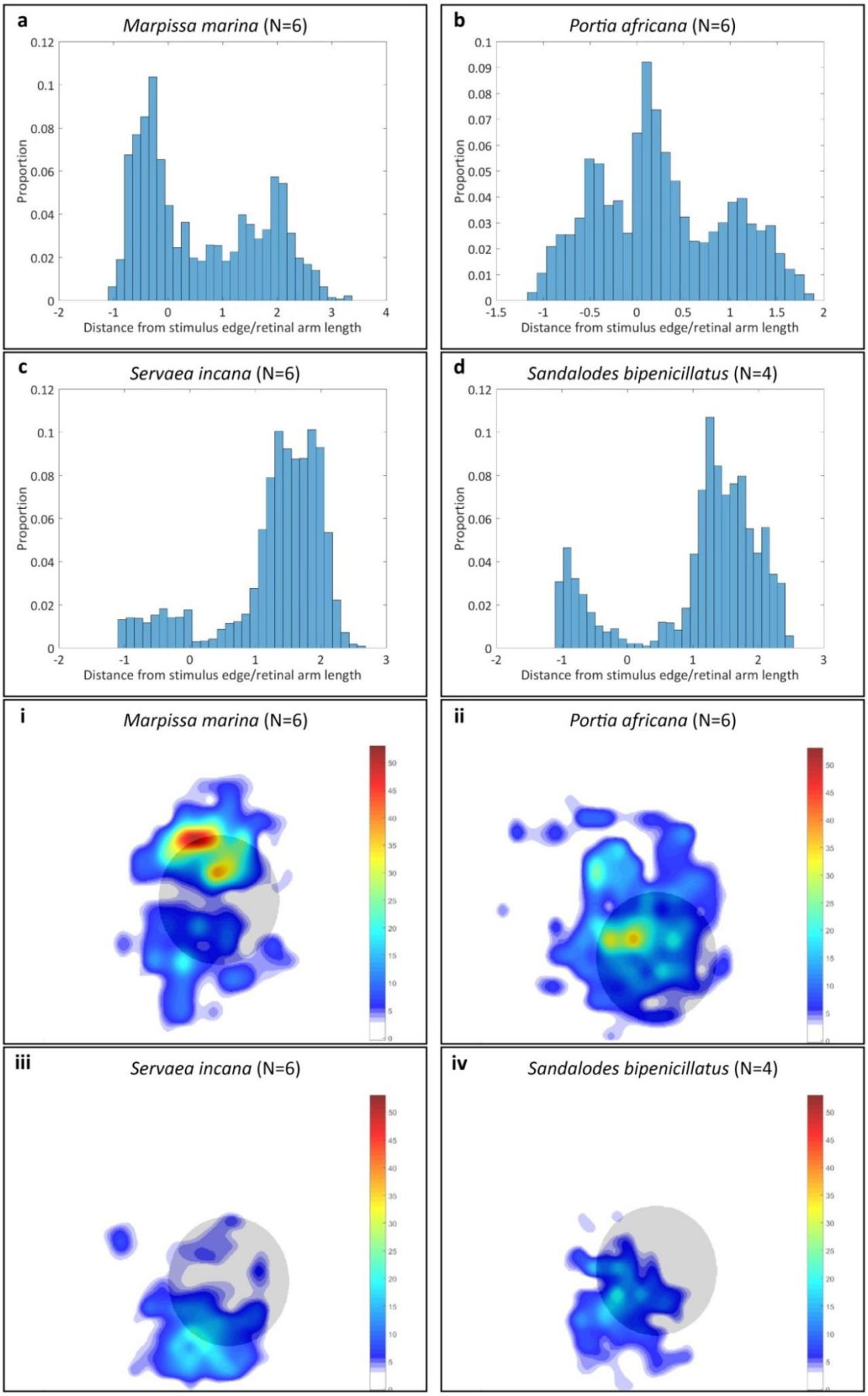

Figure 5: Distance histograms and corresponding heatmaps for the Circle stimulus for all four species. Heatmap values have been normalised to the highest value, in this case for *M. marina* (image i).

(g) *Two circles*

The histograms for this stimulus (Figure 6, a-d) differed widely, although again the heatmaps suggest that the two specialist species show more interest in the stimulus than the two generalists. For *M. marina*, the two peaks match well with the two hotspots in the heatmap, with one peak about one retinal arm length away from the stimulus, and a second one about four arm's length away. Both *S. incana* and *S. bipenicillatus* have a dip around zero, a cluster between 0-0.5 and a larger cluster with a peak around 1.7 arm lengths, for *S. incana*, and 0.7 arm lengths away for *S. bipenicillatus*. However, looking at the heatmaps, *S. incana* seems to focus mainly below the stimulus and to a lesser degree, around the inside edges, while *S. bipenicillatus* focuses on a much larger area completely engulfing the stimulus, with a large hotspot along the inner edge of one aspect of the stimulus. Finally, for *P. africana* there was a less distinct pit around 0, with a peak around 1.3 retinal arm lengths. The corresponding heatmap shows that while there is a distinct hotspot inside one of the circles of the stimulus and along its bottom edge, the range of the heatmap goes far below and to the left of the stimulus.

(h) *Eyes*

This stimulus elicited very strong responses for *M. marina, P africana* and *S. bipenicillatus*, while *S. incana*'s strongest response is only around 66% of the maximum (Figure 7i-iv). The distance histograms of *M. marina* and *P. africana* (Figure 7a-b) were rather similar, both showing a bimodal distribution with peaks at 0 and 1 retinal arm lengths from the stimulus edge (suggesting use of both the fovea and the retinal arm edge for image analysis), and another small cluster around three retinal arm lengths away, corresponding to hotspots at the bottom of the screen in the heatmaps (Figure 7i). *S. bipenicillatus* also had a bimodal distribution (Figure 7d), with one peak around 0 and the second around 1.3 retinal arm lengths from the stimulus edge, corresponding to the two hotspots bellow the stimulus in the heat map (Figure 7iv). Overall, *M. marina, P africana* and *S. bipenicillatus* seem to follow the edges of the stimulus with a wide range above and below it. In contrast, *S. incana* appeared less 'interested' in the stimulus (Figure 7c, iii), focusing mainly below the stimulus at a distance of between 1.5-2 retinal arm lengths from the stimulus.

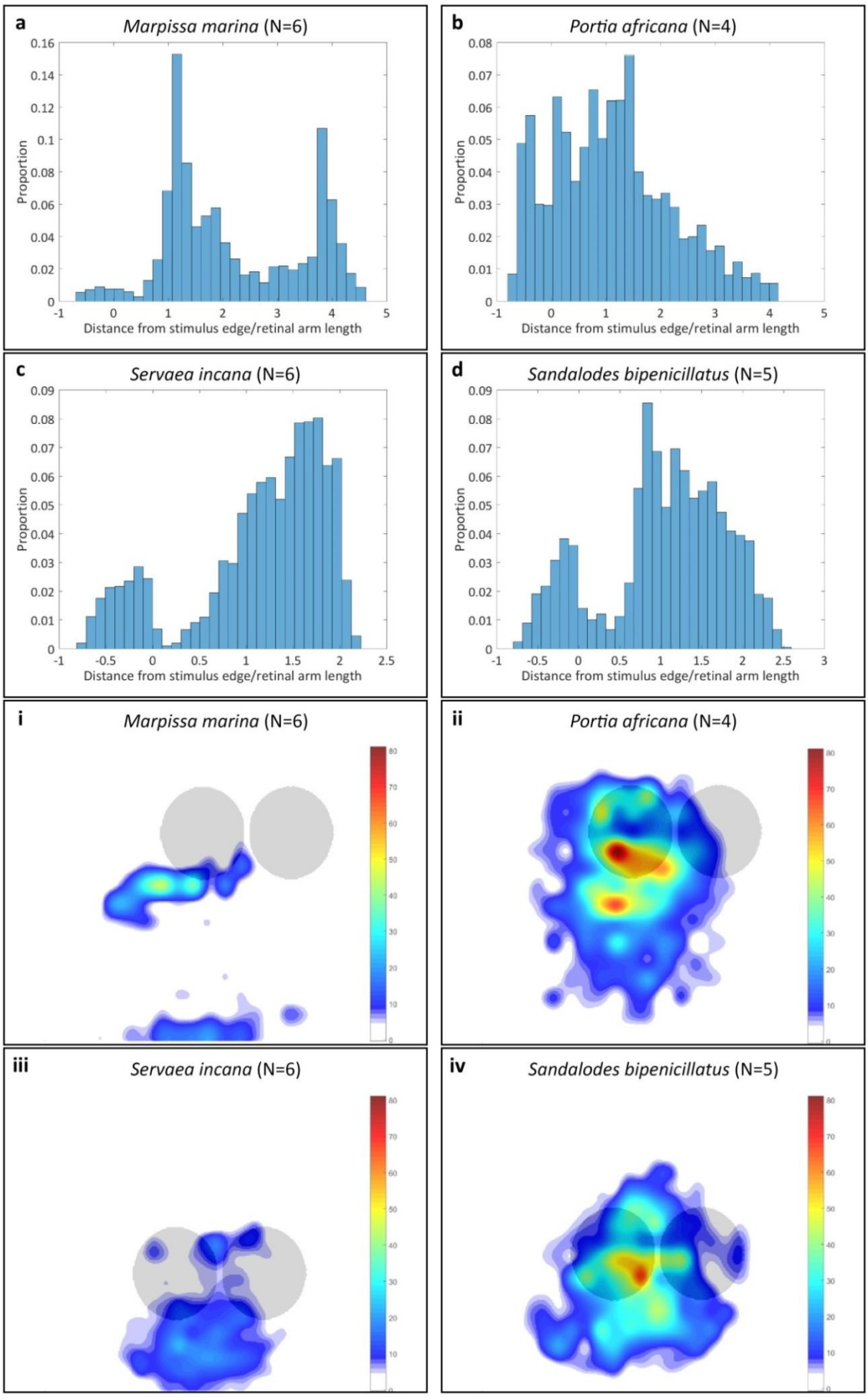

Figure 6: Distance histograms and corresponding heatmaps for the Two circles stimulus for all four species. Heatmap values have been normalised to the highest value, in this case for *P. africana* (image ii).

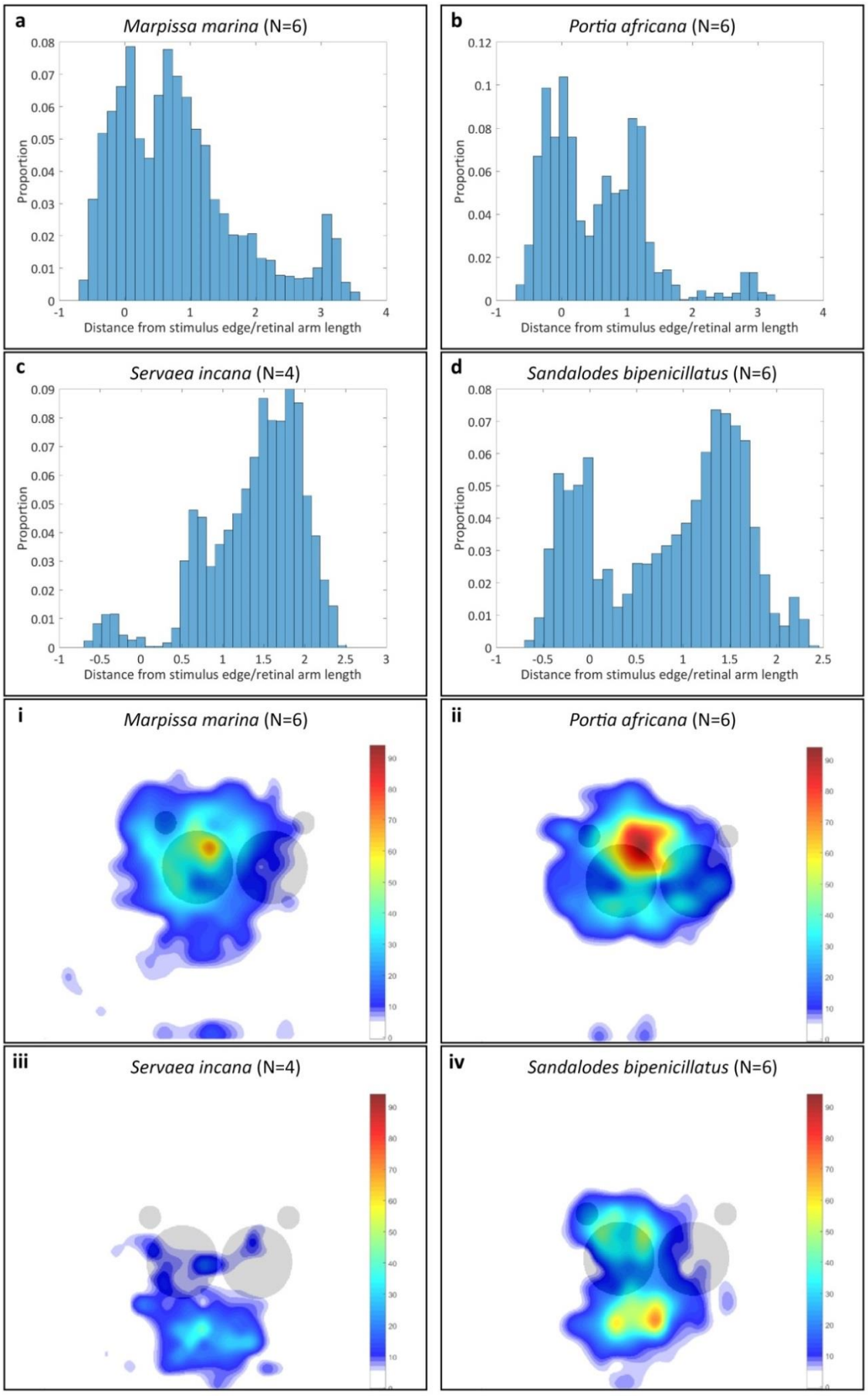

Figure 7: Distance histograms and corresponding heatmaps for the Eyes stimulus for all four species. Heatmap values have been normalised to the highest value, in this case for *P. africana* (image ii).

(i) *Salticid*

This stimulus elicited a particularly strong response in *P. africana* (Figure 8ii) compared with the other species (Figure 8i, iii, iv). Notably, all four species showed hotspots below the stimulus, as well as around the 'AL eyes' of the stimulus. For all species, the histograms peaked at around 0, although the distribution of the histograms was wider for *S. incana* (Figure 8c) and for *S. bipenicillatus* (Figure 8d) and was quite narrow in *M. marina* (Figure 8a) and *P. africana* (Figure 8b), possibly suggesting a more targeted search in the latter two species.

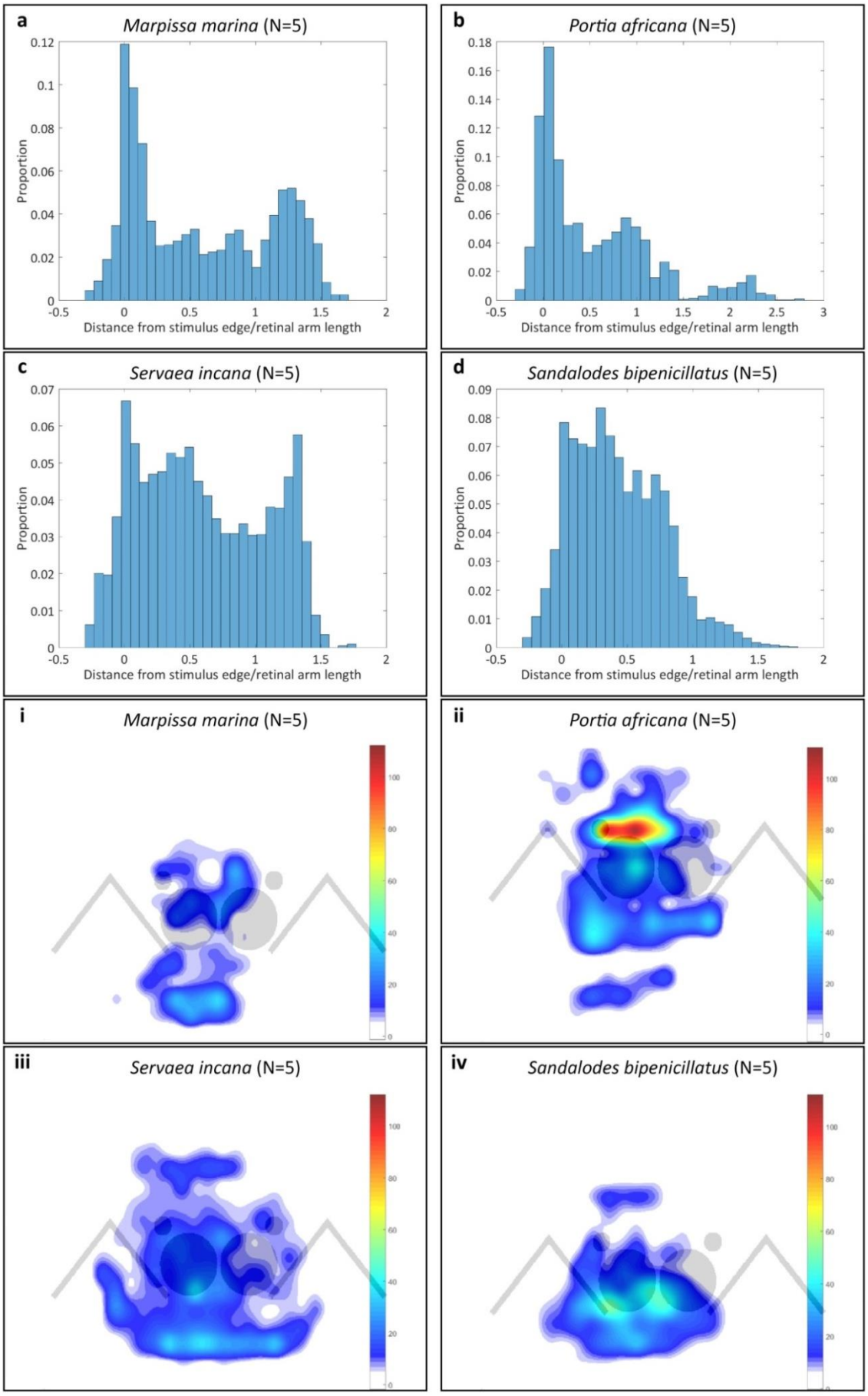

Figure 8: Distance histograms and corresponding heatmaps for the Salticid stimulus for all four species. Heatmap values have been normalised to the highest value, in this case for *P. africana* (image ii).

(j) *Within species comparisons across stimuli*

When heatmaps were normalised across each species (Figures 9-12) a few further observations can be made. For example, the heatmaps of *M. marina* all depict a tendency to focus on areas well below the stimulus (Figure 9). In *P. africana*, the stimuli that elicited the strongest interest, while differing in their level of complexity, resemble the anterior aspect of a salticid. In increasing order of strength (and of detail), these were: Two circles, Salticid eyes, and Salticid (Figure 10iv-vi, respectively). For *S. incana*, the heatmaps of the Two circles and Salticid eyes (Figure 11iv and v, respectively) were very similar in their structure, but differed in their maximal value. Another notable feature in the *S. incana* heatmaps was the vast scanning area elicited by the salticid stimulus (Figure 11vi) and what seems to be a displacement of the hottest area in each heatmap to an area around 1-1.5 retinal length (matching to the corresponding histograms, figures 3-8, image c) below the stimulus. Indeed, specifically for the Salticid stimulus, this would mean that the hot-spots may relate to the legs, the bottom of the eyes (perhaps the palps) and the top of the eyes. For the Square stimulus (Figure 11ii) the scanning pattern followed the bottom left edge. Finally, as is the case for *P. africana,* the stimuli that elicited the strongest interest among *S. bipenicillatus* (Figure 12) all resembled the anterior aspect of a salticid. As with *P. africana*, the strength of the response aligned with the level of detail in the order of Two circles, Salticid eyes, and finally the Salticid stimulus (Figure 12iv-vi, respectively), although the image of the Bar (Figure 12i) also elicited strong responses.

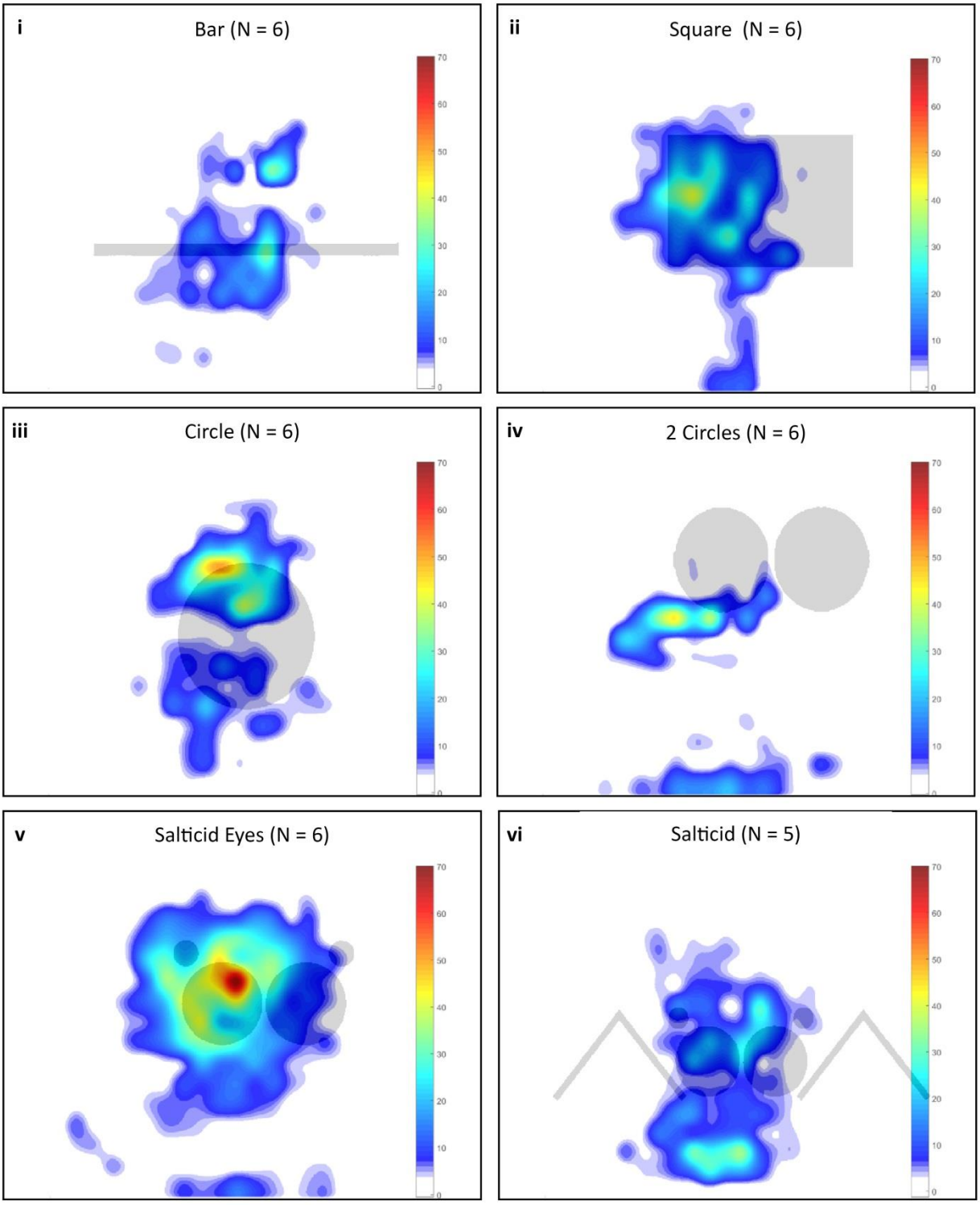

Figure 9: Scanning heatmaps of *M. marina* to all stimuli normalised to the highest value, in this case for Salticid eyes stimulus (image v).

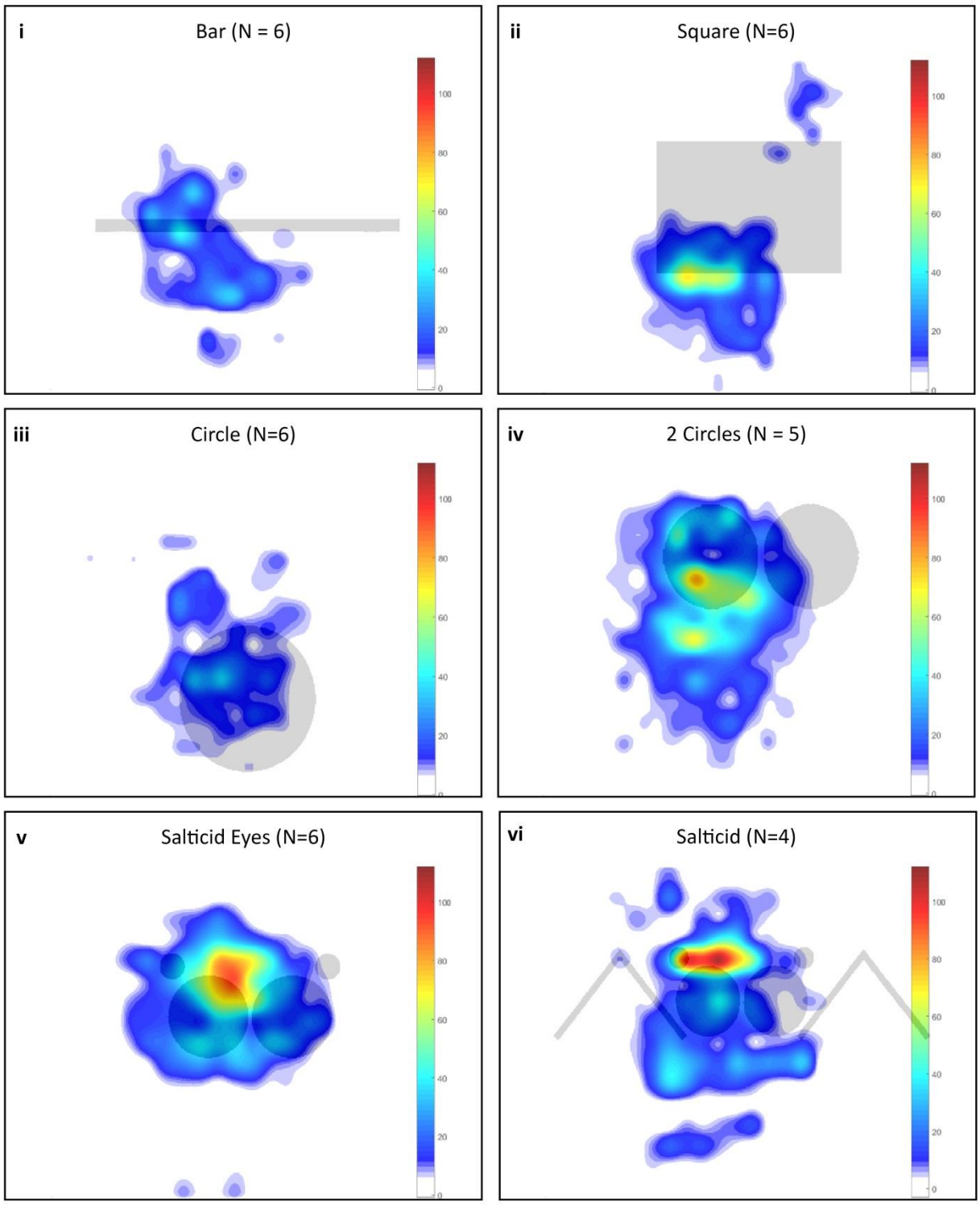

Figure 10: Scanning heatmaps of *P. africana* to all stimuli normalised to the highest value, in this case for the Salticid stimulus (image vi).

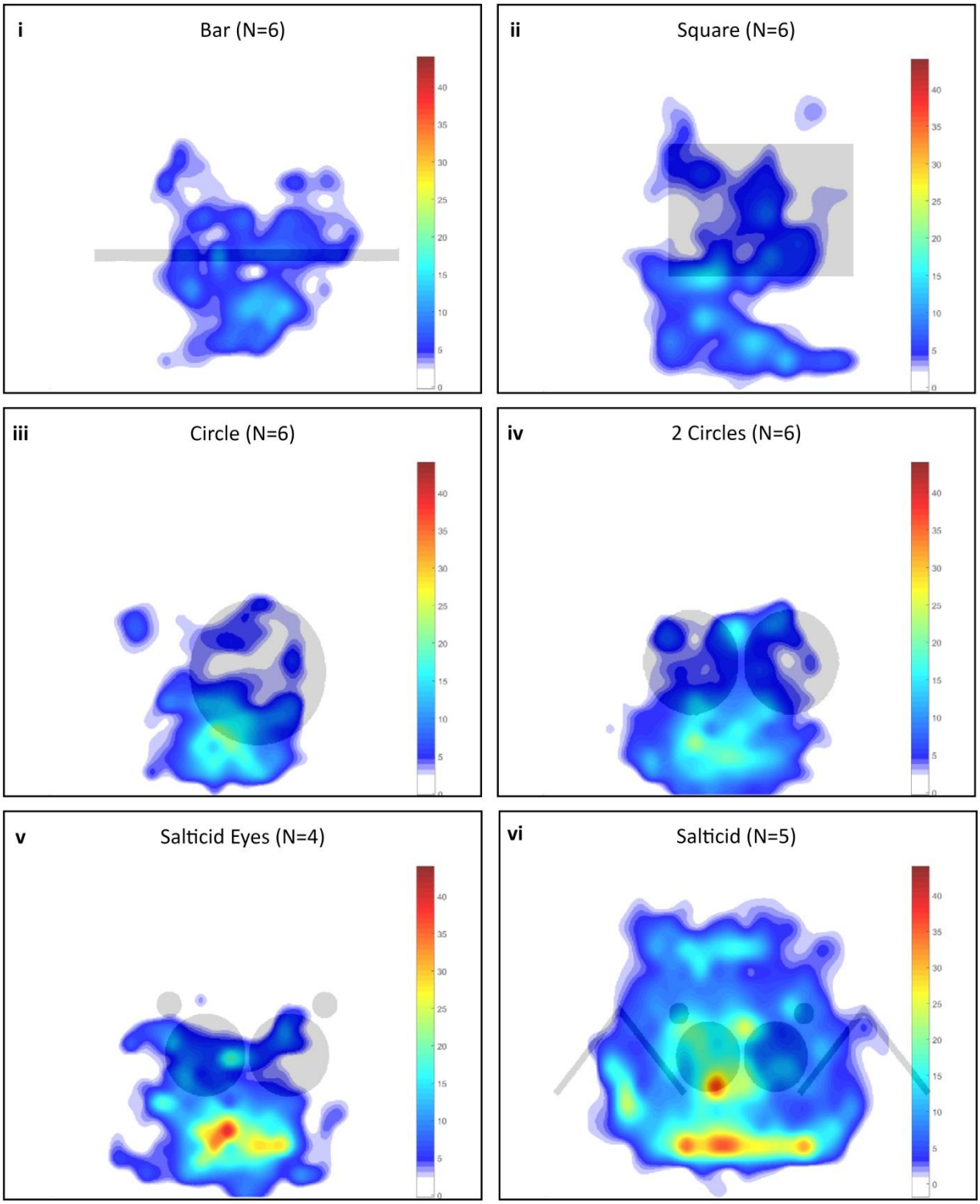

Figure 11: Scanning heatmaps of *S. incana* to all stimuli normalised to the highest value, in this case for Salticid eyes stimulus (image v).

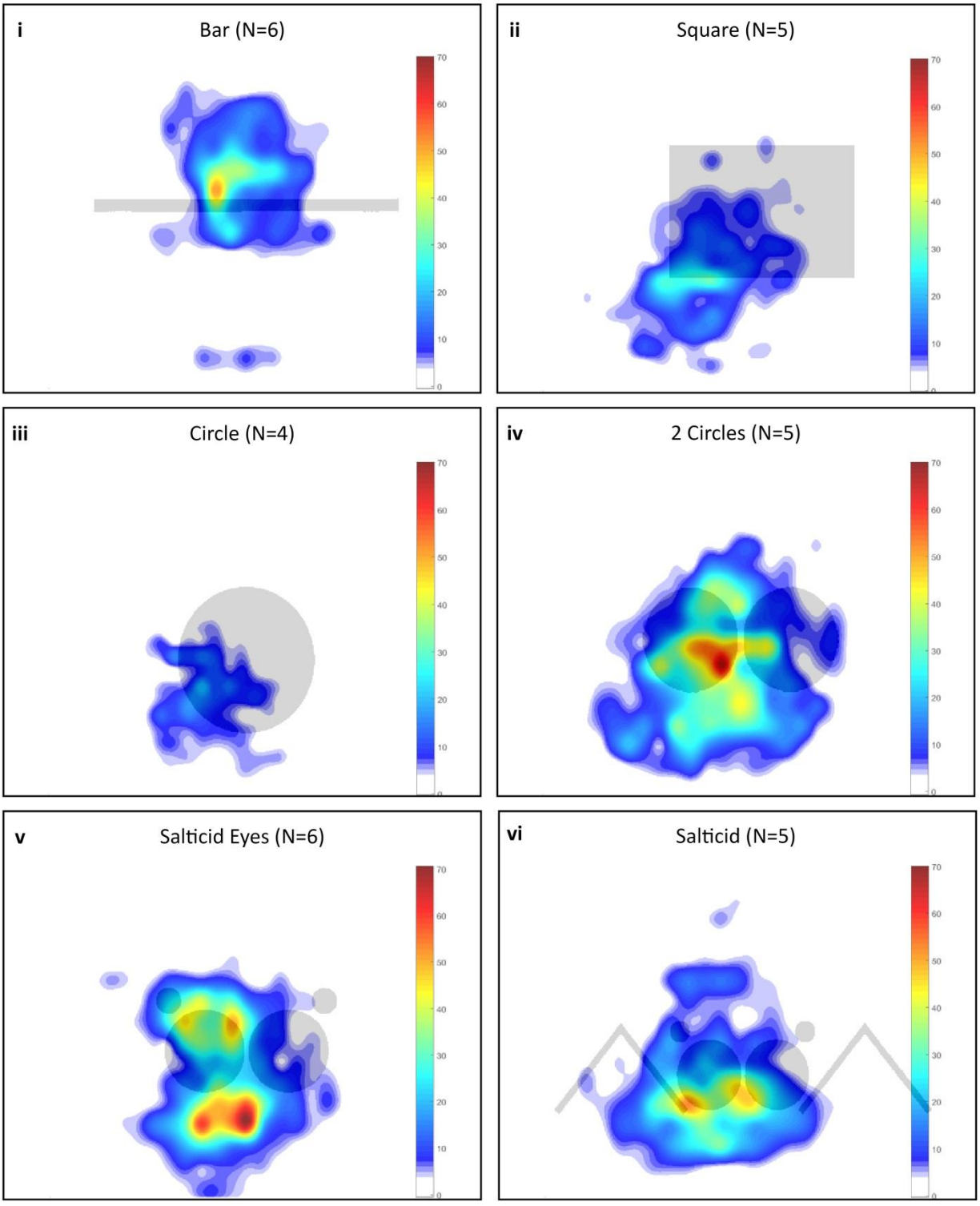

Figure 12: Scanning heatmaps of *S. bipenicillatus* to all stimuli normalised to the highest value, in this case both for the Two circles stimulus and the Salticid Eyes stimulus (images iv and v respectively).

Discussion

These data show that salticid scanning patterns are highly dependent both on the species and on the image being scanned. A particularly suggestive aspect of this work is that predatory specialisation may be associated with a more intensive visual scanning paradigm, exemplified in terms of the amount of time spent scanning (i.e., the number of frames spent scanning). In particular, predatory specialists seem to spend more time analysing images of interest, while focusing on a narrower region. Perhaps in accordance with the differences between the specialists and generalists we found that the three cases where non-significant results were found when comparing the between stimuli within species were all within the generalist predators. Moreover, all three of these (*M. marina*: Square vs. Spider, *S. incana*: Square vs. Eyes and *S. incana*: Circle vs. Eyes) are between the stimuli with little natural significance (the square and circle) and the ones with the most natural significance (eyes and salticid), suggesting that generalists don't differ as much in their scanning patterns even when the stimuli are of stronger biological relevance. Indeed, this finding is not wholly unexpected given the degree of visual detail these specialised predators take into account in their decision making (Jackson and Nelson 2012, Dolev and Nelson 2014, 2016). Nonetheless, the strong significant differences between the species (as seen when comparing within each stimulus) are likely a result of the different natural environments and life histories of these four species and any robust conclusion regarding differences in scanning behaviour between generalists and specialists evidently needs considerably more species to be investigated.

While no specific trends in scanning patterns were revealed statistically, the existence of stimulus-dependent patterns is apparent. Regardless of stimulus and species, it would seem that edge detection is an important goal of the scanning patterns. This can also be seen quite clearly when viewing the tracks themselves (see Figure 13). While extent of the edge tracking is somewhat hidden by the nature of the heatmaps, the histograms exemplify this quite nicely by the location of the peaks. These also reveal that salticids tend to use the edge of their retinae (either top or bottom arm) for edge detection. However, this could also be a result of the simple two-dimensional nature of the stimuli. Overall, both the distance histograms and the different heatmaps show that the spiders focus primarily on and around the non-moving stimulus, rather than, as Land (1969a) suggested, a sequential back-and-forth scan of the visual field. Given the AM eyes' narrow field of view, it is very likely that the AL eyes play a key role in guiding the scanning patterns of the AL eyes, giving us new insight into the roles of the lateral eyes and the foveal regions of the AL eyes (Land 1971, 1972, 1985; Zurek et. al 2010, Zurek and Nelson 2012).

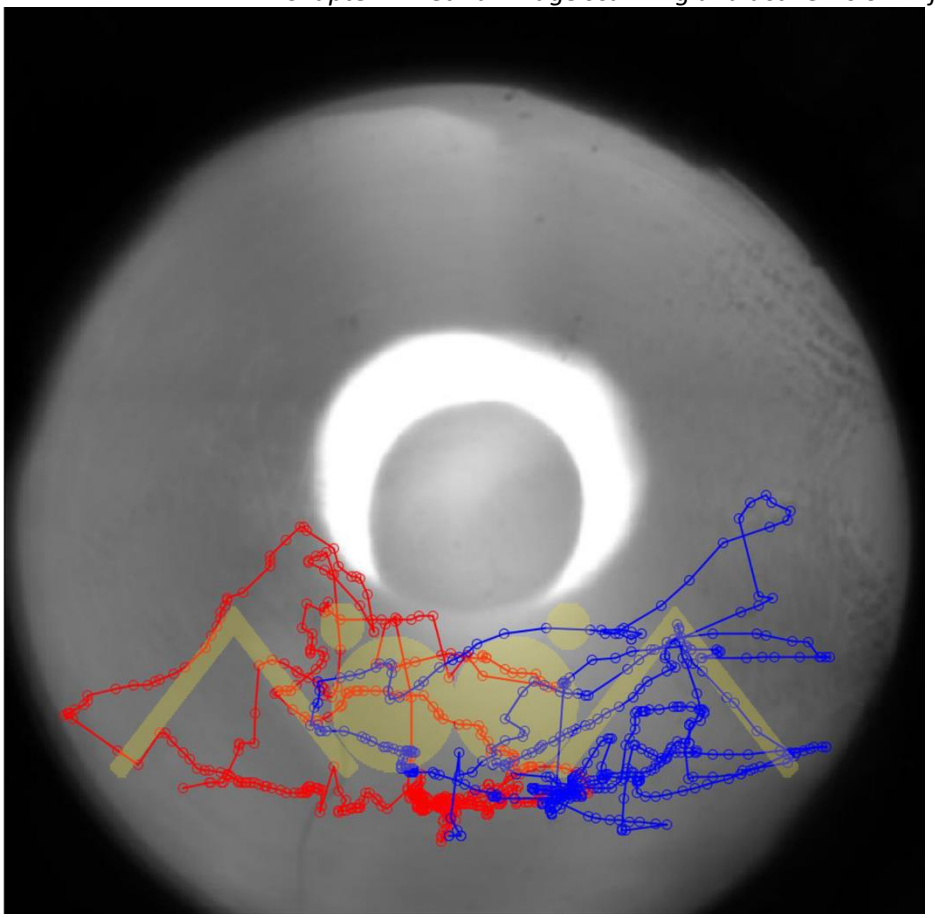

Figure 13: Scanning pattern of an example *S. incana* viewing a Salticid stimulus (colour of stimulus modified for figure). Red and blue tracks denote the different retinae. Each dot conveys a frame. Note that the scanning patterns are prior to filtering of points that did not move between consecutive frames (see methods).

Further inspection of the heatmaps also suggest some species-dependent patterns. For example, *M. marina*, in addition to analysing along stimulus edges, tend to also search well below the stimuli. *P. africana* scans of the Salticid eyes stimulus and the Salticid stimulus are very similar, while the other scanning patterns are quite different from each other. *S. incana* scanning patterns seem to be shifted around 1-1.5 retinal arm lengths below the stimulus.

It is most likely that analysis of scanning behaviours of the individual spiders, as well as separate analysis of the movement of each individual retina would reveal much more of the underlying algorithms of visual scanning. This analysis was not done here as a essential requirement for such analysis is a common starting point (i.e., fixation point) for the spider retinae from which scanning motions commence (Eckstein 2011, Chuk et al. 2014, Schurgin et al. 2014). For these experiments this common starting point was not performed.

One the most noteworthy outcomes of the study of visual search patterns in animals is its application in artificial entities, such as systems in intelligent vehicles that detect pedestrians and other obstacles (Yang and Huang 1994, Bertozzi et al. 2002, Brinkworth and O'Carroll 2009, Yoshida 2016) and

even computer-aided detection of abnormalities in medical images such as X-ray mammograms (Nagaraj et al. 2010). However, the study of eye movement in animals is full of difficulties. One of the major problems when trying to record eye movements in animals is that the equipment used is often very large and bulky, even preventing the animals from being able to move freely (Fuchs and Robinson 1966, Kimmel et al. 2012, Longordo et al. 2013, Wallace et al. 2013), or is very intrusive, often requiring operations and implants on the animal (Rodriguez et al. 2001, Schwarz et al. 2013). The latter potentially creates significant effects on the neural responses of the animal (Ravassard et al. 2013) and both the prevention of movement and invasive techniques will likely generate stress, which could have strong effects on the visual search processes of the animal. The use of salticids for eye-tracking introduces an approach which has a number of advantages over eye-tracking in other animals. Perhaps more than any other eye-tracking paradigm, this system is almost completely un-intrusive. Furthermore, while the setup is initially cumbersome, once placed in front of the eye-tracker, as far as the spiders are concerned, they are freely moving in the environment due to the polystyrene balls they hold onto. However, this does potentially create conflicting visual-proprioceptive information for the animal, as while the animal 'freely moves', the visual world doesn't move along with it. For the analyses done here, these conflicts most likely didn't alter the search-patterns performed by the spiders, as visual scanning is a process which, given the speed of retinal movements compared to the speed of spider motion, is most likely primarily performed while the spider is stationary (Forster 1977, Land 1969a, 1999). Nonetheless, addressing this issue would be most rewarding and can be achieved by coupling virtual reality systems that have recently been developed and used in salticids (Peckmezian and Taylor 2015) with the eye-tracker system. Indeed, such a set up would be incredibly informative in the study of the neurobiology of the proprioception and its relation to retinal movements, salticid navigation, cognitive maps and even problem solving.

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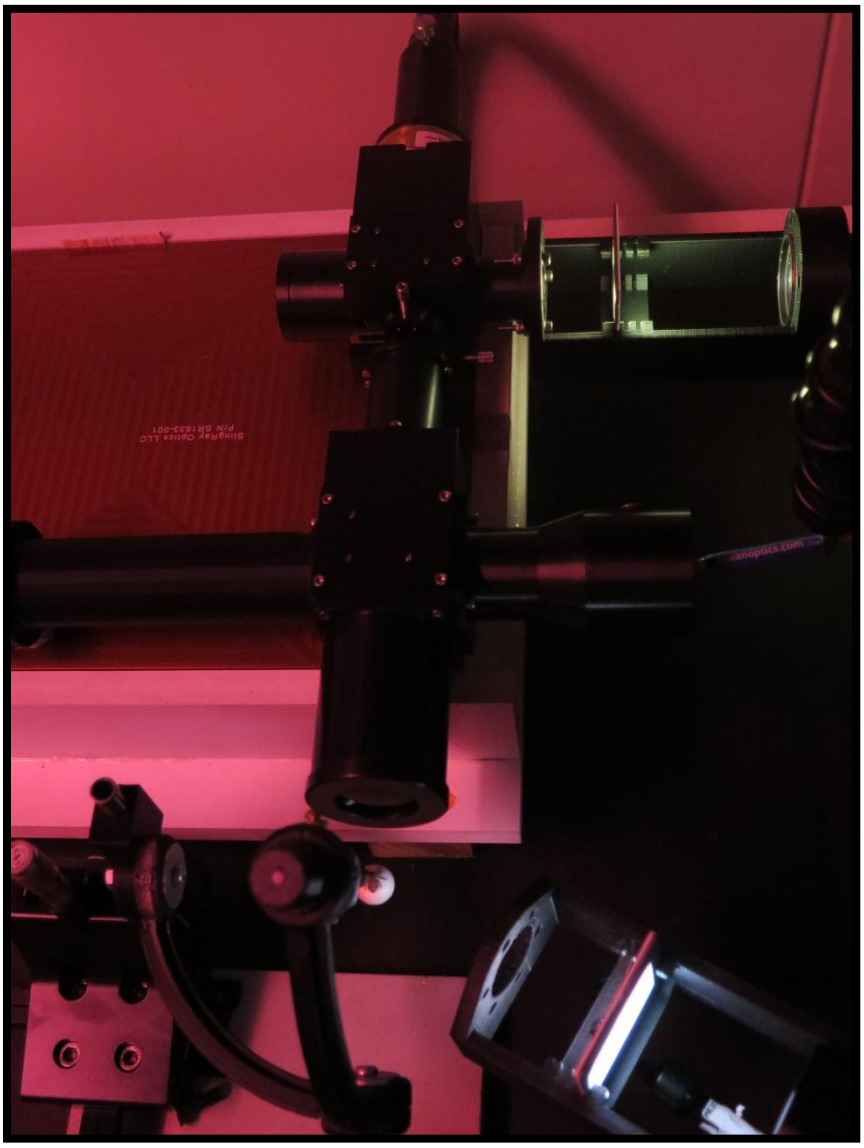
Chapter 5

Expectations and reality: The effects of visual priming on retinal scanning motion



A priming experiment in progress with S. incana at eye-tracker (Photo: Y. Dolev)

Abstract

The effects of priming on visual search patterns are well documented. However, such studies have never been done on animals with distributed visual systems. Salticids have large forward-facing camera-type eyes ('primary' eyes) which feature high resolution vision and movable retinae with which they scan their environment. Additionally, they have three pairs of smaller ('secondary') eyes that primarily act as motion detectors featuring wide fields of view which collectively encompass 360^{o}. In this work I used matching paradigms or expectation violation paradigms to test the effects of visual priming of the secondary posterior lateral eyes on the visual search patterns of the primary eye retinae of salticids. Using either a circle or a bar as a priming stimulus, a secondary stimulus – which either matched the primed stimulus or was the alternative, mismatching, stimulus - was then projected to the primary eyes. I found that priming appears to cause an initial visual search by the primary eyes for the primed stimulus before they started following the outline of the stimulus projected to the primary eyes. These effects were delayed; when priming an image for 500 ms with no inter-stimulus delay, priming had no effect in the first session but it did affect subsequent sessions. This duality was not evident when there was a 500 ms inter-stimulus delay. These data show that there are two levels of visual processing for the posterior lateral eyes: an initial rapid motion detection process, and a second process requiring > 500 ms which provides some shape-based information to the primary eyes.

Introduction

For every visual animal, the ability to rapidly assess and categorise the visual field is integral for survival. Because identification is improved based on experience, an important aspect for achieving this is through priming – an implicit memory effect in which exposure to one stimulus (i.e., the primer, being visual, olfactory or of any other sensory modality) influences the response to a subsequent stimulus (Wiggs and Martin 1998).

The effects of priming on the behaviour of animals have been studied for decades. A prime example is maternal behaviour in rodents. Early research showed how maternal behaviours are primed with exposure to olfactory and auditory cues from mice pups (Noirot 1969). Work such as this has led to our current knowledge regarding the neurobiological and endocrinological structures and processes responsible for priming maternal behaviour (Rubin and Barfield 1980, Fahrbach et al. 1984, Calamandrei and Keverne 1994, Rosenblatt et al. 1994). Another prominent example of the behavioural effects of priming is aggressiveness, where an image or a scent of a male primes aggressive behaviour in other males (mice and hamsters: Hurst 1993, Potegal and Coombes 1995; fish: Hogan and Bols 1980, Cruz and Oliveira 2015).

While the importance of priming in various cognitive tasks has been extensively researched, its role in visual search in non-human animals is relatively poorly understood. In humans and other primates, the effects of priming are usually studied by comparing the visual search patterns (where the subject is required to find a specific target) following different priming modalities, such as repeated stimuli or switching stimuli (expectancy–violation). The effects of these modalities are usually measured by their temporal aspects (i.e., priming shortens the task execution time, Maljkovic and Nakayama 1994, Maljkovic and Nakayama 1996, McPeek and Keller 2001, Becker 2008) or by the resulting bias in target selection (i.e., priming affects which target is selected by the subject, Brascamp et al. 2011, Meeter and Van der Stigchel 2013). The latter effects are referred to as attention shifts, and show that a powerful factor determining what an animal (including humans) looks for is what it has attended to previously.

Attention is often thought of as a gateway to learning and memory; however, the converse is equally true. That is, mechanisms for learning and memory play a critical role in the selection processes that determine which of the (often many) stimuli in a complex environment are attended to. These effects are commonly also referred to as priming, although it is more accurate to think of these as long-term priming. The neural bases of these mechanisms, as well as those of short-term or immediate priming are beginning to be understood (Desimone and Duncan, 1995; Desimone 1996; Schater and Buckner, 1998).

Nevertheless, this work is typically based on human or primate perception processes, with very little work done on the effects of priming on the visual processes of other animals. The exception is the use of birds under the domain of psychological studies. Using measurement parameters similar to the ones addressed above (i.e., the temporal and target selection effects), researchers have shown the effects of visual priming on the visual search patterns in pigeons (Blough 1989, Blough 1991, Blough 1992, Blough and Lacourse 1994, Fremouw et al. 1998, Ohkita et al. 2014, Gibson et al. 2015) and blue jays (Bond and Kamil 1999, Goto et al. 2014). Visual search patterns and object categorisation are also affected by priming, including not only in single sense priming (e.g., visual priming in pigeons: Blough 1991; in rats: Tafazoli et al. 2012), but also cross-modal priming, where different sensory modalities are engaged (e.g., barn owls: Hazan et al. 2015). Cross-modal priming has also been investigated in jumping spiders (Salticidae), where a mosquito-eating species visually identifies its preferred prey more quickly when it smells that particular prey and vice versa (Cross and Jackson 2009). Salticids have also been used to investigate the behavioural effects of the role of 'representation' in working memory (Cross and Jackson 2014), which can be tested using expectancy violation methods. In the visual sensory domain, these methods typically present the test subject with a given scene which then disappears from view and the individual's time spent looking at the restored scene is measured. If the scene has not been altered this would be an expectancy-confirmation test, but if it has been altered this constitutes an expectancy violation (Shettleworth 2009). These experiments provide the basis for further investigation into the mechanisms underlying visual priming.

The salticid visual system is a distributed visual system, comprised of four pairs of simple (camera-type) eyes. In addition to a single, large, forward-facing pair of eyes (principal or Anterior Median (AM) eyes), this is composed by three smaller pairs of eyes: the forward facing anterior lateral (AL) eyes, the lateral-facing posterior median (PM; reduced in most species), and the rear-facing posterior lateral (PL) eyes (Land 1985). These latter three pairs of eyes (AL, PM, PL) are collectively known as 'secondary eyes' and jointly encompass c. 360^{o} field of view, with considerable binocular overlap in the fields of view of the more forward-facing AL eyes, but only minimal overlap between the AL and PL eyes (see Appendix 2, Figure S1; Land 1971, Land 1972, Zurek et al. 2010, Zurek and Nelson 2012). These eyes are fixed to the carapace and the retinae have no freedom of motion. While the AL eyes possess a fovea and convey some additional spatial information (Forster 1979, O'Carroll 1989, Zurek et al. 2010, Zurek and Nelson 2012), collectively the secondary eyes largely act as motion detectors, with movement detected in their field of view causing a rapid optomotor response which results in the target being acquired by the AM eyes. The AM eyes have a narrow boomerang-shaped retina, subtending about 20° vertically by 1° horizontally in the

central region, which is roughly six receptor rows wide (Land 1972). These eyes provide outstanding spatial acuity (as low as 0.04°) over a narrow field of view of 3-5° (Land 1969a,b, Williams and McIntyre 1980, Blest et al. 1990).

The visual search patterns of most animals consist of a combination of saccades and fixations resulting in a series of stationary retinal images, rather than continuous retinal image motion (Land 1999). However, perhaps due to their unusual morphology, salticid visual search patterns differ significantly from that of most other animals. The retinae of the AM eyes, which lie at the end of a long innervated 'eye tube', are capable of vertical, horizontal and even rotational (torsional) movement (Land 1969a). These degrees of freedom create distinct types of complex movements that can scan up to c. 28° from the central body axis. These movements not only compensate for the narrow field of view of the AM eyes, but are responsible for the detailed analysis of the spatial features of a visual scene. Rather than a visual search pattern consisting of saccades and fixations, the visual search pattern of salticids is a continually scan of the outside world with their two primary eyes, resulting in a continually changing retinal image (Land 1969a, Land 1999). Indeed, at least four distinct kinds of movements can be distinguished from the salticid retinae: (i) spontaneous activity, (ii) saccades, (iii) tracking motion and (iv) scanning movements (Land 1969a).

This study concentrated only on the saccadic and scanning motions. Salticid saccadic motions are analogous to human saccadic eye-movements, whose goal is to bring a target seen in the periphery (in the case of salticids, in the field of view of the secondary eyes) on to the fovea, for which the salticid equivalent lies in the AM eye retina. Saccades are a very rapid movement of at least 15° within 0.1 s (Land 1969a) and are often accompanied by a rapid rotation by the spider. Following a saccade, the retinae then either start scanning motions or they return slowly to their resting position (Land 1969a). Scanning motions are stereotypical and largely follow the edges of the objects of interest in the visual field (see Chapter 4). In Chapter 4, the typical scanning motions of salticids were examined by presenting the spiders with simple stimuli directly in front of them. Consequently, these stimuli were visible only to the AL and AM eyes. Here, the effects of expectancy violation and expectancy conformation priming on scanning movements in salticids were investigated. This was done by, prior to presentation of a visual stimulus to the AM eyes, either the same stimulus or a different one was presented to the PL eyes (which do not share a field of view with either the AL or the AM eyes). In addition, the PL eyes were primed at different intervals prior to presentation to the AM eyes to explore the effects of time on evidence of priming on the subsequent AM eye scanning routines. Specifically, I predicted that animals primed with one stimulus would subsequently

scan with their AM eyes for that stimulus, and that this would be clear from search patterns involving violation of expectations. I also predicted that this effect would be more marked at long interval durations due to perceptual processing, possibly involving more neural recruitment for the task over time.

Methods

Test spiders were adult female *Servaea incana* (N = 36) that were starved for 3-5 days prior to testing. All spiders were kept in a temperature-controlled laboratory set to 24^{o}C - 26^{o}C, with a photoperiod of 12L:12D (lights on at 07:00). Spiders were housed individually in enriched 1 litre cylindrical transparent plastic jars, as described in Zurek et al. (2010). Spiders were fed 1-3 house flies (*Musca domestica*) and a variety of field caught dipterans (mass equivalent to c. 1 house fly) weekly. All tests were carried out between 08:00 and 14:00.

The retinal movements of the primary eyes of each spider were recorded while the spiders were presented with a visual priming stimulus of either a bar or a circle to the left PL (LPL) eye, followed by presenting the same or an alternate stimulus to the AM eyes. Only the LPL could be used because of space constraints that prevented the placement of a projector on the right side of the test spider. The spider eye-tracker used in this study is the same as in the previous chapter (see Appendix 2). In addition to the equipment used in Chapter 4, an additional projector and spider theater (the priming projector, Figure 1) were placed behind the test spider so as to project an image onto the LPL eye. Six different priming paradigms were tested with N = 6 spiders in each. The priming paradigms (Figure 2) consisted of priming the LPL eye with either a bar or a circle for 500 ms, followed by either immediately (no time interval between the PL stimulus and the AM stimulus) showing the AM eye a stimulus (bar or circle) showing the AM eyes a stimulus (bar or circle) after a 500 ms delay (this was only done when the second stimulus was different to the primed stimulus). These stimuli were chosen as to keep the stimuli as simple as possible while maintaining a strong difference between them. For simplicity, the two timing paradigms shall be referred to as 'Immediate' and 'Delayed'. The 'main stimulus', which was presented to the AM eyes, was presented until 1 min from the start of the test (i.e., stimulus presentation to the PL eye) had elapsed (i.e., either 59.5 or, in the case of violation expectation tests, 59 s). This constituted one iteration. After the first iteration, there was a 2 min interval and iteration 2, which replicated iteration 1, was then presented to test spiders.

Spiders were tethered in place using dental micro-applicators dabbed in bees wax and then attached to the spiders' cephalothorax. The spider was then placed within a micromanipulator, allowing

fine positioning of the spider in front of the eye-tracker (see Appendix 2, Figures S3, S6 and S7). A polystyrene ball (diameter of 10-15 mm, depending on the size of the spider) was placed under the spider for it to freely walk on. While the balls were heavier than the spiders, they were light enough for the spiders to hold onto and turn easily, and no signs of fatigue were evident (see Zurek et al. 2010). As a result, any movements attempted by the spider result in the polystyrene ball moving (rather than the spider). Sessions began once a clear image of the AM retinae was obtained and the retinae came to a resting position. Each session was 60 s long.

For simplicity, only the left retina in each video was tracked in each video. Tracks were created using the ImageJ MTrackJ plugin. For each frame, the centre of the fovea (the point in the middle of the two arms of the retina) was marked as a point in the track. Tracking started around 3 s before the priming stimulus presentation. Tracking ended when the retina either stopped moving or after 15 s. Prior to analysis, tracking points where the retina did not move (i.e., identical points) were identified and removed using Matlab. The resulting tracks were split into three (for the immediate priming paradigms) or four (for the delayed priming paradigms) sections: prior to priming (spontaneous activity; depicted in yellow in all figures), during priming stimulus (saccadic motions appeared within this section; depicted in red in all figures), during the inter-stimulus-interval (visual searching motions; only for the delayed priming paradigms; depicted in purple in relevant figures) and during the main stimulus presentation (scanning motion; in all figures depicted along a time gradient from blue to green). Sessions in which the primed stimulus in either the first or second iteration did not evoke a saccadic AM eye motion or where no scanning motions followed the saccadic movement were discarded from analysis because without the saccadic motion (or scanning) it was impossible to determine whether the priming stimulus was in fact perceived.

I ran a K-means cluster analysis on the scanning motions from each track, using k=1. This is a data-partitioning algorithm, also known as Lloyd's algorithm (Lloyd 1982), that assigns n observations (the x,y coordinates of the tracks) to exactly one of k clusters defined by centroids, such that the sum of the distances between the n observations and the assigned centroid is minimised. This analysis resulted in the distance (squared Euclidean distance, in pixels) from the cluster centroid for each point in the track (i.e., centroid distance) giving a unified measure of the dispersion of the scanning motions in the form of the mean centroid distance (MCD). These were then compared between the first and second iteration for each spider using paired t-tests. In the cases where the df were below 100 (due to short bouts of movement) the t-tests were re-run with a bootstrapping paradigm of 1000 repetitions. Additionally, paired tests were

run on the pooled data for the number of frames in which there was retinal movement ('active frames') for the matched and expectancy violation tests in order to assess the overall activity levels between iteration 1 and iteration 2, as well as unpaired tests on the 'active frames' between the second iteration of the Immediate priming paradigm and both iterations of the Delayed priming paradigms. Statistical and cluster analyses were performed using SPSS v20, GraphPad Prism v6 and Matlab R2015. The specific analyses used for each experiment are detailed below.

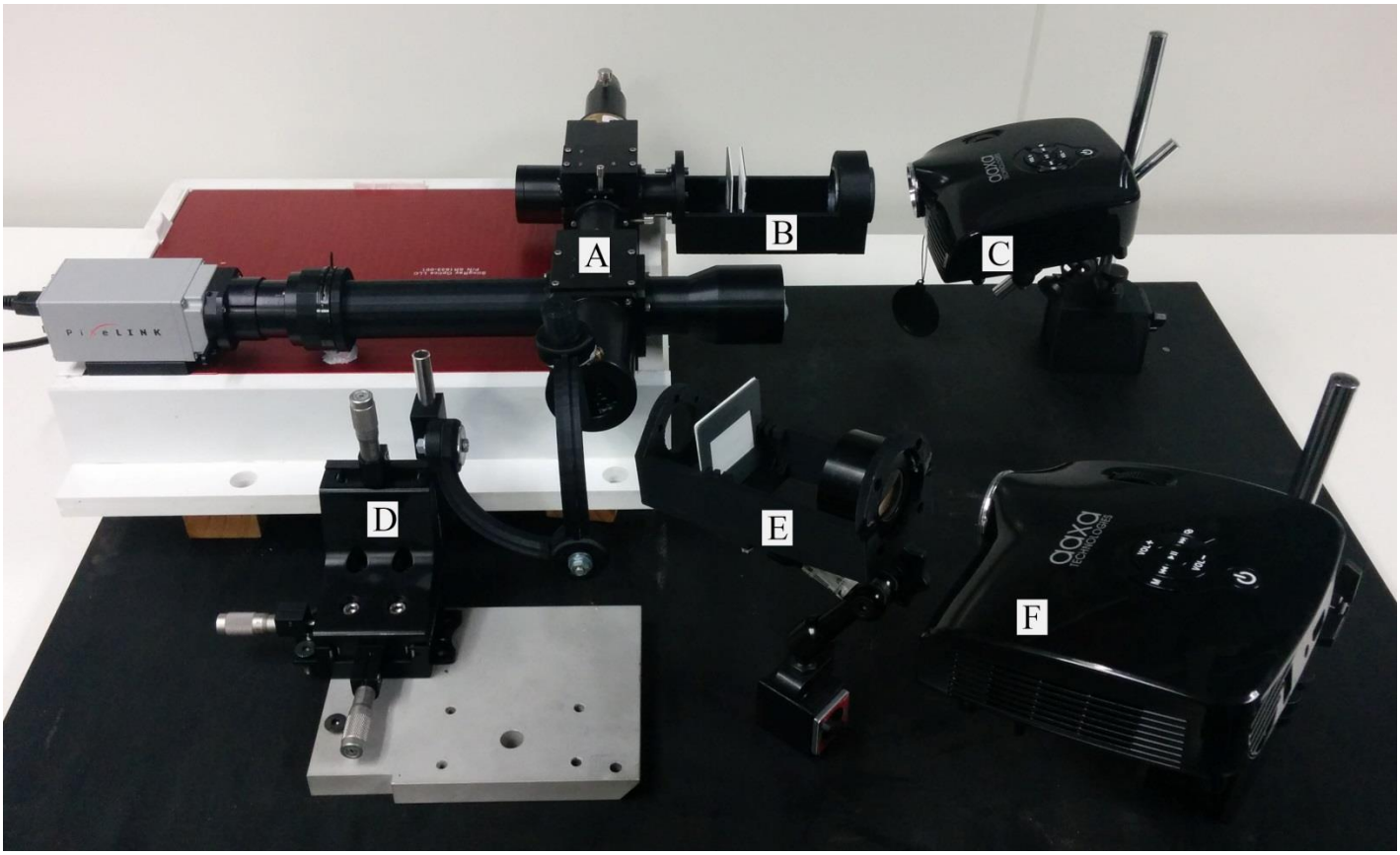


Figure 1: Eye-tracker setup for the priming experiment. A. the eye-tracker with the IR camera on the left (grey) and the near IR light source at the back. B. The spider theatre for the main stimulus projected on to the AM retinae by the mini projector at C. D. The micromanipulator for fine placement of a tethered salticid in front of the eye-tracker. E. the spider theatre for the priming stimulus projected on to the spider's left PL eye by the mini projector placed at F.

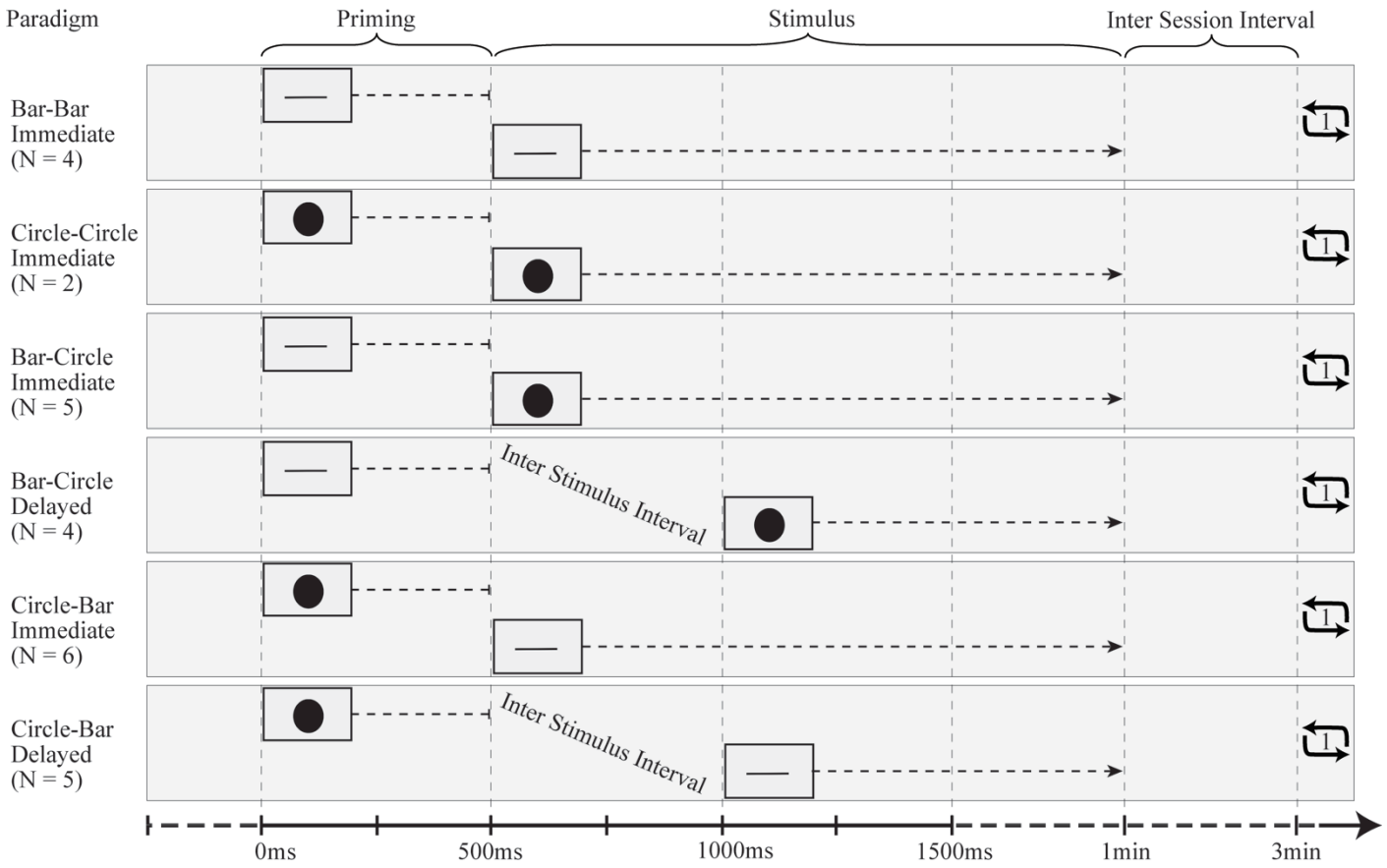


Figure 2: The six different priming paradigms used in this study involving two stimuli: either a bar or a circle. Each paradigm was repeated twice (first and second iteration). Priming stimulus duration was always 500 ms. After this, the stimulus presentation to the AM eyes was either immediately after presentation of the primed stimulus (Immediate) or after a 500 ms inter-stimulus interval (Delayed). The number of valid cases (out of six, see Methods) is stated on the left.

Results

While six spiders were tested for each priming paradigm, some cases were not analysed, either because no saccadic motion was triggered by the stimulus or because no scanning of the stimulus by the AM eyes ensued the saccadic motion. All valid retinal tracks are displayed in Figures 3-8 and the results for the different paradigms are summarised in Tables 1-6.

For the stimulus matching paradigms (where the primed and main stimulus were identical), all spiders showed significantly smaller mean centroid distance (MCDs) in the second iteration compared with the first (Tables 1,2), suggesting a 'tighter' spread around the main stimulus (Figures 3, 4). However, the total number 'active frames' (see methods) for each valid test spider did not differ between iteration 1 (median, 25^{th} and 75^{th} percentiles, respectively = 252.5, 192.5 and 312.8) and iteration 2 (median, 25^{th} and 75^{th} percentiles, respectively = 239.5, 198.5 and 299.5) of the pooled Bar-Bar (N = 4) and Circle-Circle (N = 2) tests (Wilcoxon-matched pairs test, W = 11, p = 0.293), suggesting similar levels of retinal 'activity'.

Visual inspection of Figures 3 and 4 suggest very different tracking routines to the bar, which were largely horizontal movements, and the circle, which appeared to be largely circular movements. While the clustering of the tracking was significantly smaller in the second iteration (Tables 1,2), there was no apparent change in scanning 'behaviour' within a single iteration, as indicated by the colour-coding of the scanning routine over time for each test.

Table 1: Bar - Bar Immediate priming paradigm
The mean centroid distance (MCD) for each iteration of each spider under the Bar-Bar Immediate priming paradigm (Figure 2) and the results of the t-test comparing them. The centroid distance is the squared Euclidean distance (in pixels) from the center point of the cluster of track points to each point in the track.

Spider	Iteration 1 MCD	Iteration 2 MCD	t	df	p
a	4148	3037	2.59	136	< 0.05
b	4510	3645	3.07	253	< 0.005
c	7402	2273	15.9	252	< 0.001
d	2642	1346	4.425	218	< 0.001

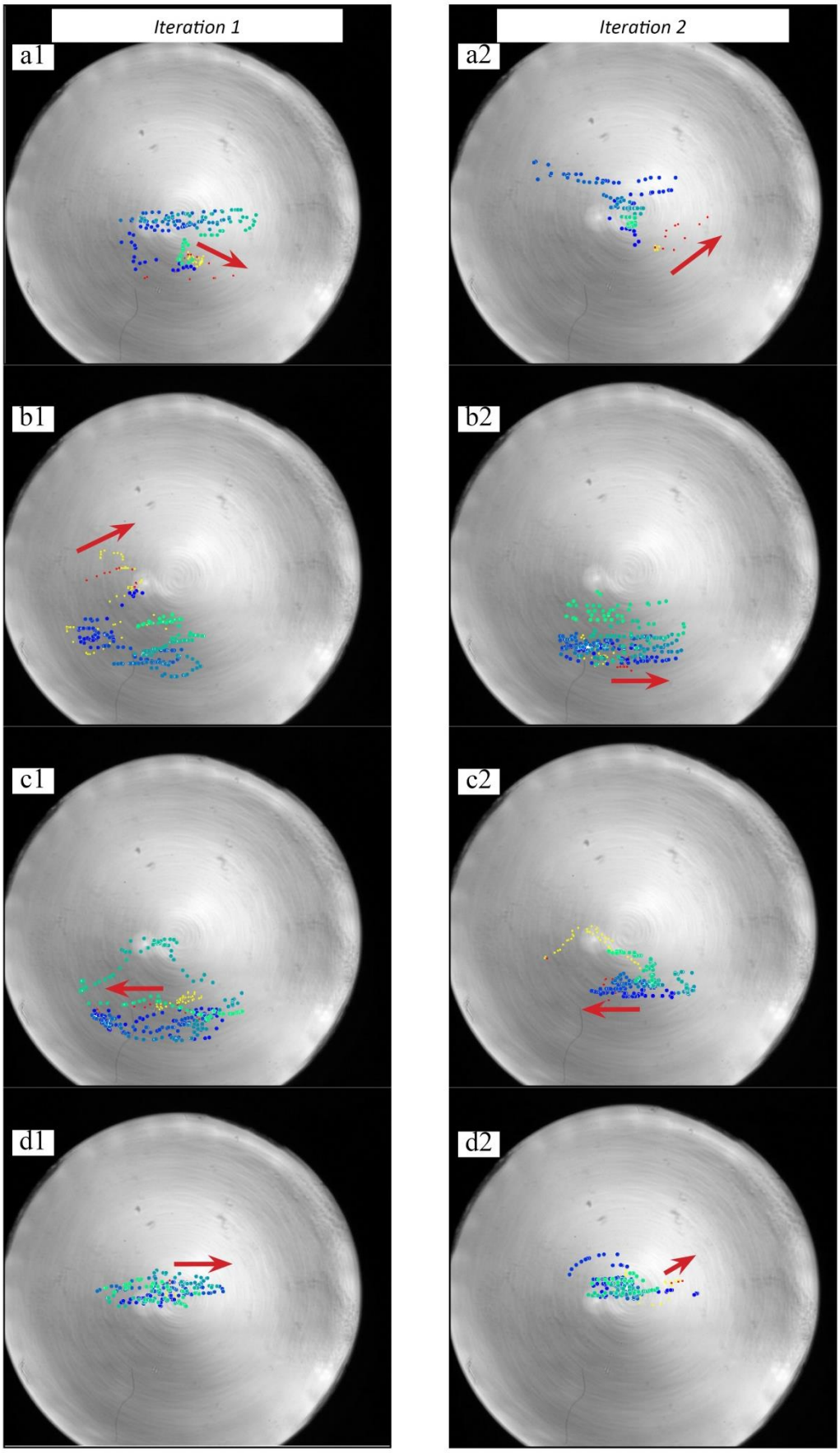


Figure 3: Tracks from the salticid AM eye retina in the Bar-Bar Immediate priming paradigm. Only the left retina was tracked. Each point relates to the centre of the fovea, the point between the two arms of the retina. Yellow: spontaneous activity, prior to stimuli presentation. Red: movement during the presentation of the priming stimulus showing characteristic saccadic motions (note back and forth saccadic motion due to the spider rotating its body to face the stimulus); Red arrows depict initial direction of saccade. Dark-blue to light green: visual scanning motions during the main stimulus presentation. Colours change from blue to green over time. Spider ID and iteration labelled in each panel.

Table 2: Circle - Circle Immediate priming paradigm
The mean centroid distance (MCD) for each iteration of each spider under the Circle-Circle Immediate priming paradigm (Figure 2) and the results of the t-test comparing them. The centroid distance is the squared Euclidean distance (in pixels) from the center point of the cluster of track points to each point in the track.

Spider	Iteration 1 MCD	Iteration 2 MCD	t	df	p
a	10841	7156	5.06	201	<0.001
b	6154	5004	3.12	363	< 0.005

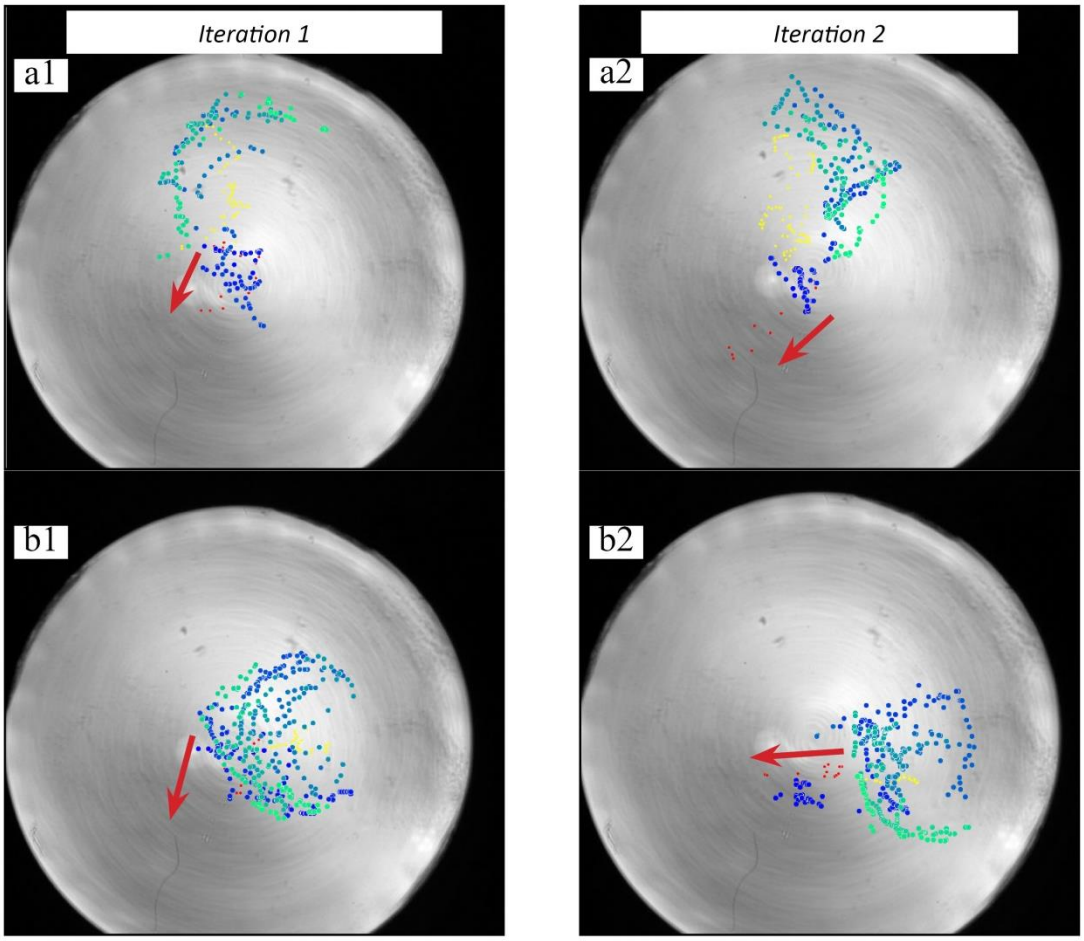


Figure 4: Tracks from the salticid AM eye retina in the Circle-Circle Immediate priming paradigm. Only the left retina was tracked. Each point relates to the centre of the fovea, the point between the two arms of the retina. Yellow: spontaneous activity, prior to stimuli presentation. Red: movement during the presentation of the priming stimulus showing characteristic saccadic motions; Red arrows depict initial direction of saccade. Dark-blue to light green: visual scanning motions during the main stimulus presentation. Colours change from blue to green over time. Spider ID and iteration labelled in each panel.

As in the matching paradigm tests, under the Circle-Bar Immediate priming paradigm, significant differences in the MCDs were found between the first and second iteration in all spiders, but differed in directionality. Unlike in the matching paradigm tests, here the MCD in the first iteration was smaller than that of the second iteration in all but one of the six spiders (Table 3, spider e). These distributions can be seen in Figure 5. In the track of the first iteration of spider e (Figure 5, e1) the last section of the track (bright green) the spider's retina moved out of the eye-tracker's field of view (the grey circle on which the tracks are displayed). It is this section of the track that is most likely the cause of MCD of the first iteration being larger. Paired t-tests on the normally distributed pooled 'active frames' data for each valid test spider from Bar-Circle and Circle-Bar tests showed a significant difference between iteration 1 and iteration 2 in the 'Immediate' paradigm ($t = 3.347$, $p = 0.007$, $df = 10$; means $\pm$ SEM for iteration 1 and 2, respectively: 187.7 ± 19.7 and 334 ± 46), but not in the 'Delayed' paradigm ($t = 1.238$, $p = 0.251$, $df = 8$; means $\pm$ SEM for iteration 1 and 2, respectively: 401.8 ± 51.3 and 341.3 ± 35.4). These 'active frame' comparisons match the results of the MCD comparisons, showing that under the Immediate paradigms, the spiders were significantly more active (i.e., more retinal movements in the given time period) in the second iteration than the first, while under the Delayed paradigms, no such differences were apparent. These differences again strengthen the finding that the priming paradigm has a delayed effect on the search patterns. Interestingly, when comparing the 'active frames' between the second iteration of the Immediate paradigms, and the first ($t = 0.985$, $p = 0.338$, $df = 10$) and second iteration ($t = 1.1218$, $p = 0.904$, $df = 18$) of the Delayed paradigms, no differences were found, suggesting that priming has a constant effect in terms of the probability of retinal movement (i.e., the effects of delayed priming are similar to the 'compound' effects visible only in iteration 2 in the Immediate priming paradigm).

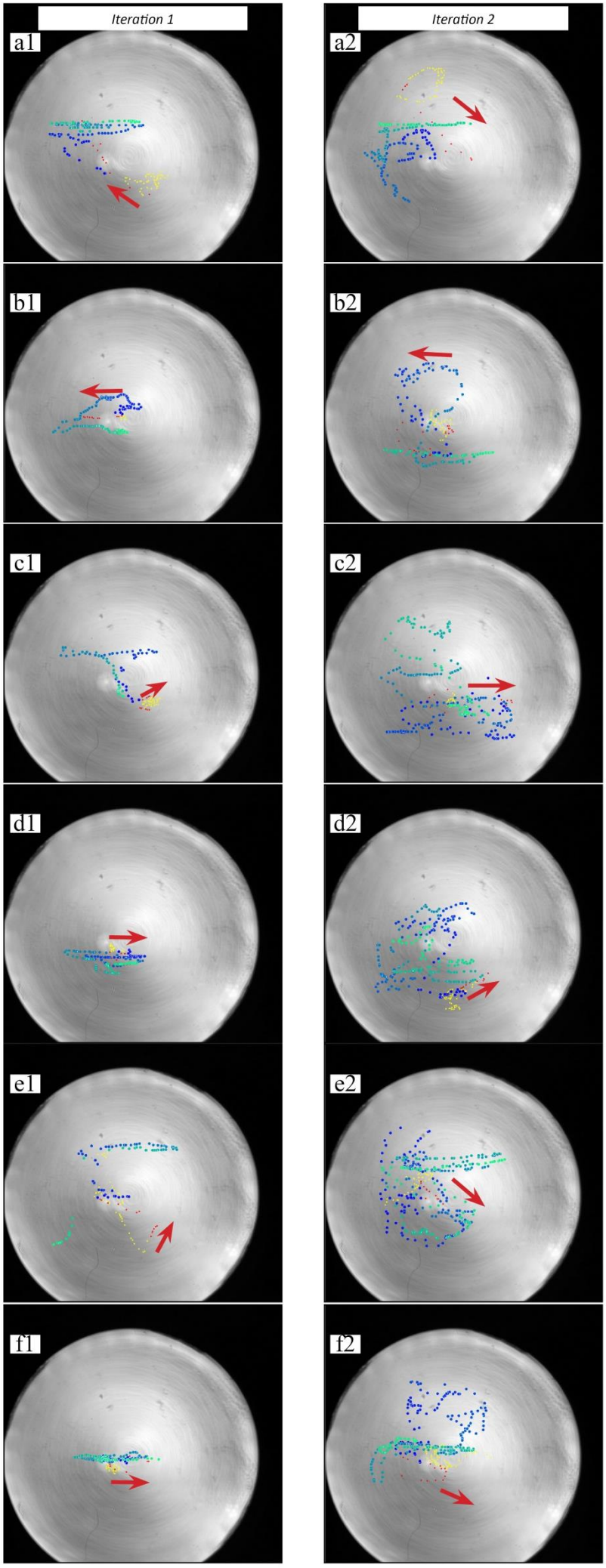
Visual inspection of Figure 5 clearly shows a larger spread in tracks in the second iteration. What is more noteworthy is that the scanning routines for the first iteration appear to be primarily horizontal, as if searching for a bar, while the scanning routines in the second iteration seem to begin in a circular fashion (as depicted by the bluish dots), and only after time changed to primarily horizontal motion. Overall, this suggests that the primed stimulus takes time to have an effect regarding what the AM eyes will search for, and if the 'expected' stimulus is not present, only then do the AM eyes appear instead to attend to the stimulus which is actually present (in this case a bar, as depicted by the green dots).

Table 3: Circle - Bar Immediate priming paradigm
The mean centroid distance (MCD) for each iteration of each spider under the Circle-Bar Immediate priming paradigm (Figure 2) and the results of the t-test comparing them. The centroid distance is the squared Euclidean distance (in pixels) from the center point of the cluster of track points to each point in the track.

Spider	Iteration 1 MCD	Iteration 2 MCD	t	df	p
a	4397	5623	-0.35	131	< 0.001
b	3221	11756	-12.82	153	< 0.001
c[a]	4741	15042	-5.19	27	< 0.001
d	2522	8546	-17.14	140	< 0.001
e[b]	15112	10655	-7.05	27	< 0.005
f	2056	8390	-12.63	157	< 0.001

a. Bootstrap using 1000 repeated measures, original df = 79.
b. Bootstrap using 1000 repeated measures, original df = 81; note outliers on bottom left of iteration 1, see Figure 5, e1 vs e2.

Figure 5: Tracks from the salticid AM eye retina in the Circle-Bar Immediate priming paradigm. Only the left retina was tracked. Each point relates to the centre of the fovea, the point between the two arms of the retina. Yellow: spontaneous activity, prior to stimuli presentation. Red: movement during the presentation of the priming stimulus showing characteristic saccadic motions; Red arrows depict initial direction of saccade. Dark-blue to light green: visual scanning motions during the main stimulus presentation. Colours change from blue to green over time. Spider ID and iteration labelled in each panel. Session c1 and e1 had fewer than 100 track points. Note bottom right track in e1 (light green) – this section of the track is the result of the spider's retina drifting outside of the field of view, from where it didn't return until after the end of the test.


Iteration 1
Iteration 2
a1
a2
b1
b2
c1
c2
d1
d2
e1
e2
f1
f2

In contrast to the Circle-Bar Immediate paradigm, under the Circle-Bar Delayed priming paradigm (Table 4), in only two out of the five spiders were significant differences found in the MCDs between the first and second iteration. In both cases (Table 4, spiders a, c) the MCD in the second iteration was larger than in the first iteration. However, in both iterations of these tests the spiders generally appeared to search widely and in a somewhat circular fashion (Figure 6), again suggesting that priming of visual stimuli to the AM eyes by the PL eyes takes time.

Table 4: Circle - Bar Delayed priming paradigm
The mean centroid distance (MCD) for each iteration of each spider under the Circle-Bar Delayed priming paradigm (Figure 2) and the results of the t-test comparing them. The centroid distance is the squared Euclidean distance (in pixels) from the center point of the cluster of track points to each point in the track.

Spider	Iteration 1 MCD	Iteration 2 MCD	t	df	p
a	7688	9460	-5.82	261	< 0.001
b	7025	7435	-0.57	286	0.57
c	6089	9223	-7.07	248	< 0.001
d	8255	8013	1.43	352	0.153
e	7761	7193	1.17	213	0.243

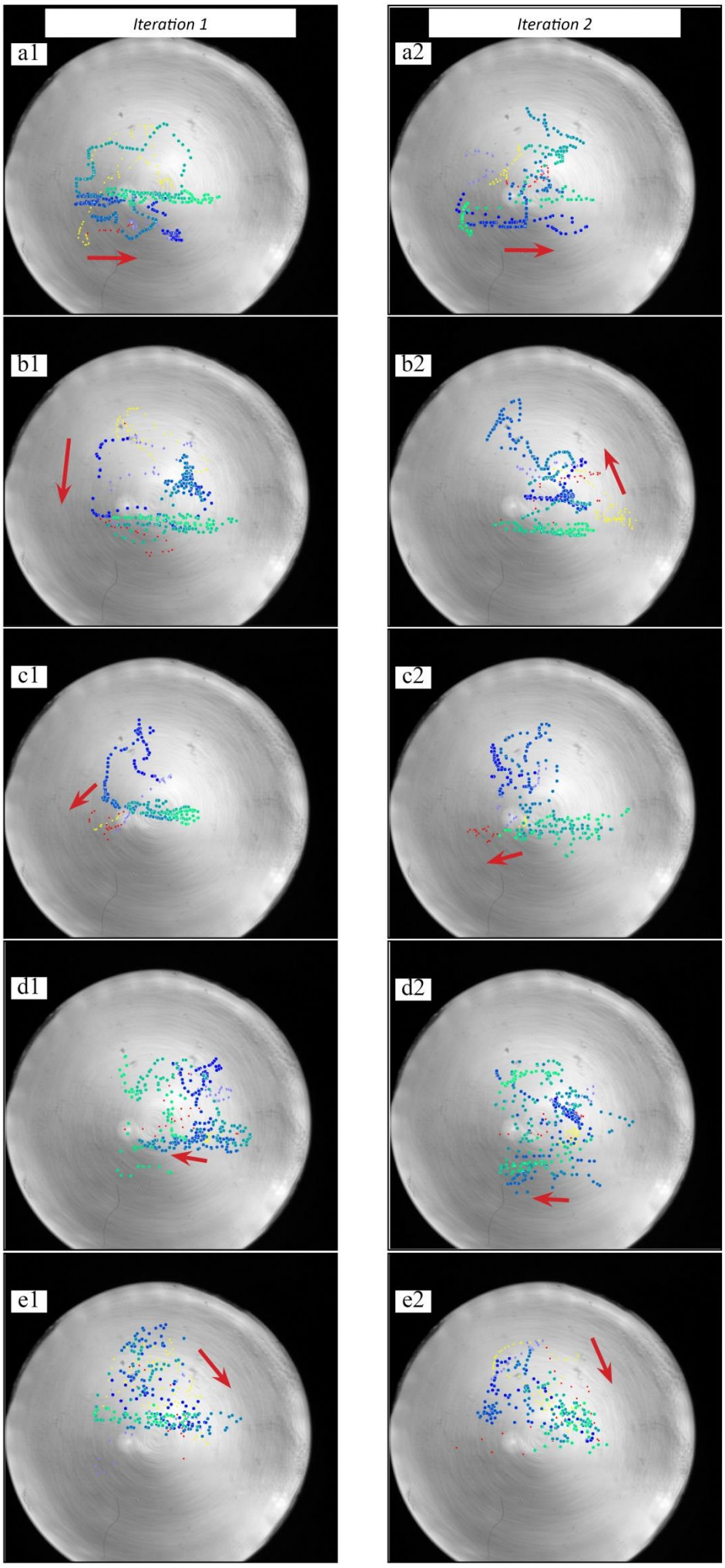


Figure 6: Tracks from the salticid AM eye retina in the Circle-Bar Delayed priming paradigm. Only the left retina was tracked. Each point relates to the centre of the fovea, the point between the two arms of the retina. Yellow: spontaneous activity, prior to stimuli presentation. Red: movement during the presentation of the priming stimulus showing characteristic saccadic motions; Red arrows depict initial direction of saccade. Purple triangles: tracks made during the inter-stimulus-interval; note lack of significant movement during these periods. Dark-blue to light green: visual scanning motions during the main stimulus presentation. Colours change from blue to green over time. Spider ID and iteration labelled in each panel.

The final two paradigms were the Immediate and the Delayed Bar-Circle tests. Under the Bar-Circle Immediate priming paradigm four out of the five spiders showed significant differences in the MCDs between the first and second iterations (Table 5). In two of these (spiders b and c), the MCD was larger in the first iteration than in the second, while in the other two (spiders d and e) the MCD in the first iteration was smaller than in the second (a trend in this direction was also evident in spider a). Under the Bar-Circle Delayed priming paradigm (Table 6) only in one out of the four spiders showed a significant difference in the MCD between the first and second iteration, with the MCD in the first iteration being smaller than that of the second iteration (spider b, Table 6).

As implied by these results, visual inspection of these tracks is less clear regarding the timing of visual inspection for the bar or for the circle, and perhaps suggest that due to the size of the circle, this stimulus may be more heavily attended (or trigger a wider-searching scanning routine) than the bar, regardless of timing between tests.

Table 5: Bar - Circle Immediate priming paradigm
The mean centroid distance (MCD) for each iteration of each spider under the Bar - Circle Immediate priming paradigm (Figure 2) and the results of the t-test comparing them. The centroid distance is the squared Euclidean distance (in pixels) from the center point of the cluster of track points to each point in the track.

Spider	Iteration 1 MCD	Iteration 2 MCD	t	df	p
a	8472	9176	-1.89	280	= 0.06
b	9605	6519	6.9	271	< 0.001
c	9906	3690	12.08	119	< 0.001
d	2729	4792	-6.794	170	< 0.001
e	4226	8801	-6.681	241	< 0.001

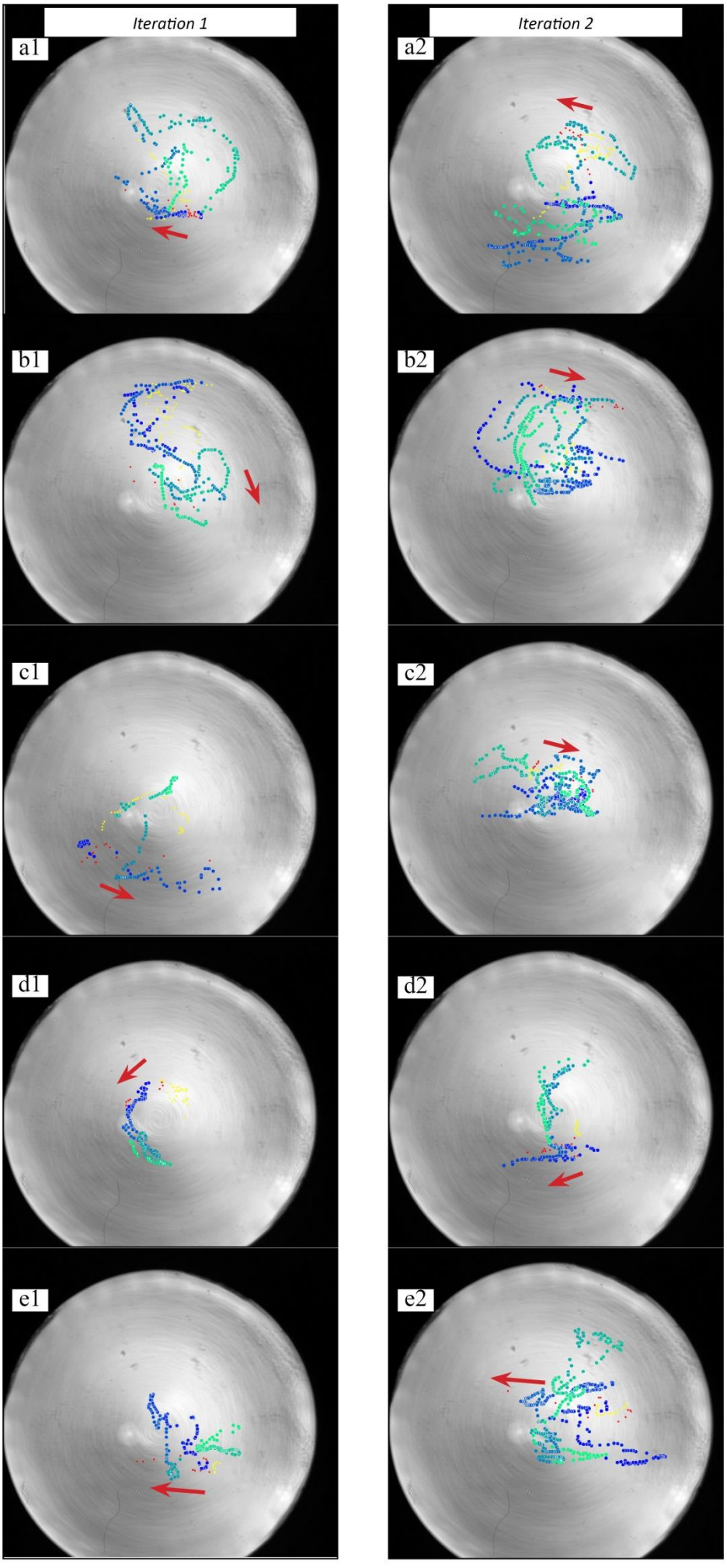


Figure 7: Tracks from the salticid AM eye retina in the Bar-Circle Immediate priming paradigm. Only the left retina was tracked. Each point relates to the centre of the fovea, the point between the two arms of the retina. Yellow: spontaneous activity, prior to stimuli presentation. Red: movement during the presentation of the priming stimulus showing characteristic saccadic motions; Red arrows depict initial direction of saccade. Purple triangles: tracks made during the inter-stimulus-interval; note lack of significant movement during these periods. Dark-blue to light green: visual scanning motions during the main stimulus presentation. Colours change from blue to green over time. Spider ID and iteration labelled in each panel. Note that the differences between the first and second iteration are less obvious in theses tracks.

Table 6: Bar - Circle Delayed priming paradigm

The mean centroid distance (MCD) for each iteration of each spider under the Circle - Bar Delayed priming paradigm (Figure 2) and the results of the t-test comparing them. The centroid distance is the squared Euclidean distance (in pixels) from the center point of the cluster of track points to each point in the track.

Spider	**Iteration 1 MCD**	**Iteration 2 MCD**	**t**	**df**	**p**
a	6618	6376	0.532	268	0.595
b	2578	4721	-9.7	499	<0.001
c	8887	8027	1.524	440	0.128
d	7339	7987	-0.88	371	0.379

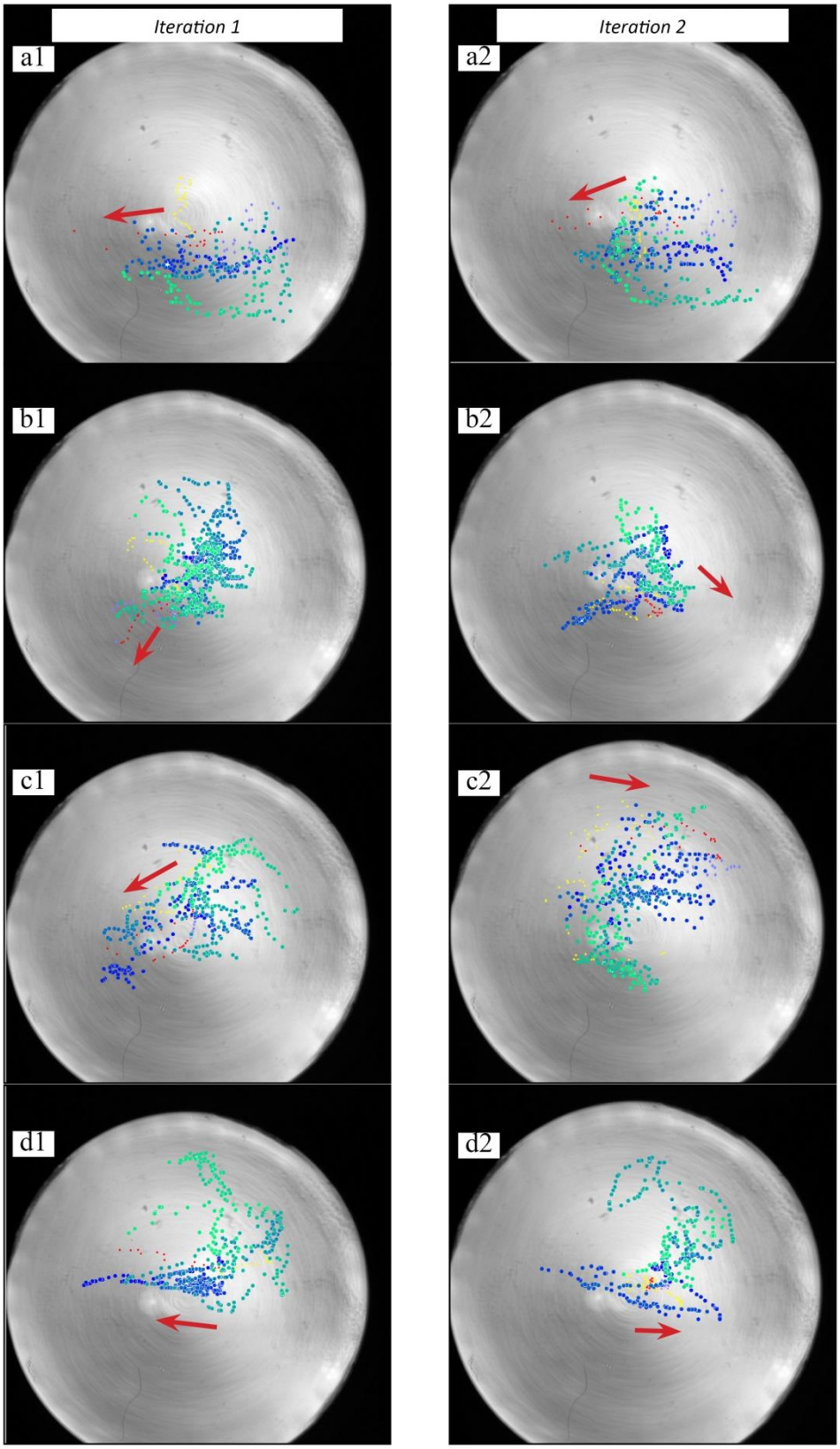


Figure 8: Tracks from the salticid AM eye retina in the Bar-Circle Delayed priming paradigm. Only the left retina was tracked. Each point relates to the centre of the fovea, the point between the two arms of the retina. Yellow: spontaneous activity, prior to stimuli presentation. Red: movement during the presentation of the priming stimulus showing characteristic saccadic motions; Red arrows depict initial direction of saccade. Purple triangles: tracks made during the inter-stimulus-interval; note lack of significant movement during these periods. Dark-blue to light green: visual scanning motions during the main stimulus presentation. Colours change from blue to green over time. Spider ID and iteration labelled in each panel.

Discussion

The results of these experiments clearly show that visual priming affects the processes of visual search, while giving us a glimpse into a number of cognitive processes pertaining to visual perception in salticids. Perhaps the most obvious of these effects is revealed by the repeated presentation of matching stimuli. In both the expectancy-confirmation paradigms (where the stimuli were the same) the breadth of visual search patterns were reduced in the second iteration. This suggests two possible, yet opposing, underlying perceptual processes. The first possibility is that in the second iteration the spiders are more focused on the visible stimulus, as is exemplified by a narrower visual search pattern. This is akin to diminished response time in visual search tasks (Huang et al. 2004, Geyer et al. 2006). Alternately, it is possible that the spiders 'lost interest' or habituated to the task and as a result we see a decrement in the search pattern.

A similar response decrement was reported by Land (1969) where due to repeated visual stimuli the bouts of retinal movement had decreased over time, suggesting it was either due to habituation or a spontaneous change in responsiveness. However, while the MCD was typically smaller in the second iteration of expectancy-confirmation, the 'activity' *per se* of the retinae in each iteration were not significantly different, arguing against the latter hypothesis.

When considering these options, it is important to remember that the visual field of the AL eyes of jumping spiders completely overlaps that of the AM eyes (Land 1971, Land 1972, Zurek et al. 2010, Zurek and Nelson 2012). In other words, the spiders perceive the presented stimulus in some detail without the use of the primary eyes (Zurek et al. 2010). In this sense, the AM eye retinal movements are a type of visually-guided behaviour, and it can be suggested that one of the likely tasks of the AM eyes is acquiring additional minute spatial details in the visual field, which are most likely directed by the AL eyes. Therefore, a narrow search pattern in the second iteration of a repeated stimulus could be an indicator of the working memory of the spiders confirming that little additional details are needed (Baddeley 2012).

Working memory varies considerably between species. In dogs, working memory has been shown to be retained for at least 240 s (Fiset et al. 2003), while in some insects this is only about 8 s (Zhang et al. 2005). While not directly tested, in salticids, working memory seems to be retained for at least 90 s (Cross and Jackson 2014), and in some species there is evidence that - with a strong enough drive (i.e., their preferred prey) - they can retain working memory for over 17 min without further reinforcement (Jackson and Wilcox 1993).

Nonetheless, given that under the Circle-Circle Immediate paradigm only two of the six spiders tested responded in both iterations, with three of the four failed sessions due to the spiders not showing any visual search patterns in the second iteration (even though they did show a saccade, Figure 9), it is possible that the processes resulting in a narrower breadth of visual search patterns may be simply that of a lowered motivation towards repeated stimuli. Testing this with further repetitions at different time intervals to determine the levels of 'active search' (see Chapter 4) as well as the breadth of the scanning patterns over time, while introducing a form of 'dishabituating' stimulus might shed some insight into these processes.

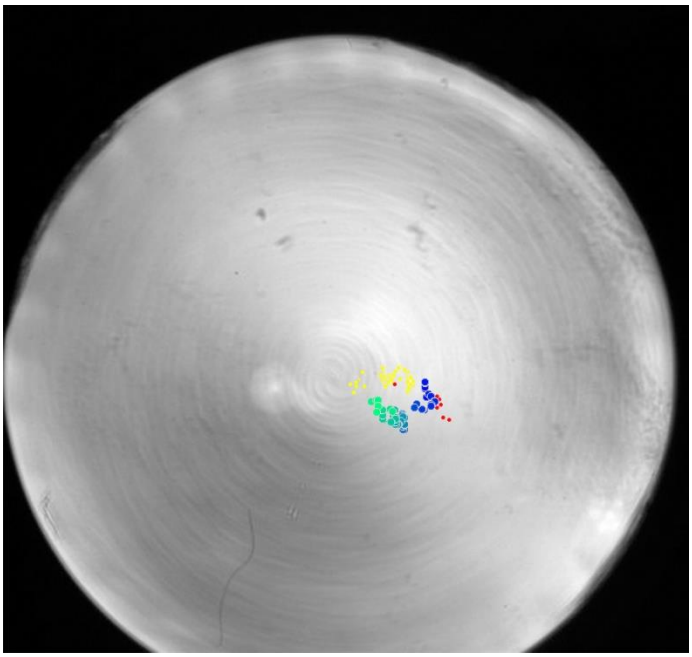


Figure 9: A failed track from the second iteration of the Circle-Circle Immediate priming paradigm. A slight saccade (in red) is apparent, although no retinal scanning commences following it.

The clear differences in the search patterns between Circle-Circle and Bar-Bar, as well as the obvious similarities within these paradigms allowed us to explore the effects of visually mismatching (expectancy violation) paradigms. Perhaps of most interest (and certainly the easier of the two mismatching paradigms to interpret) are the mismatching Circle-Bar paradigms. The stand out difference between the Immediate and the Delayed datasets is that while in iteration 1 in both tests the PL eye 'experienced' the circle priming stimulus for 500 ms, it was only in the Delayed tests that circle-like search patterns were evident. This suggests that priming can occur within 500 ms, but it takes longer than that to

have an effect on the AM eye scanning routines. This interpretation is further corroborated by the comparisons of the 'active frames', or retinal activity in the mismatching paradigms. No differences were found between the two iterations of the Delayed paradigms, but strong differences between the two iterations of the Immediate paradigm. These differences may simply be because of the 'computational' time (Kirchner and Thorpe 2006, Chittka and Spaethe 2007) taken due to the number of synapses and muscular coordination involved (Reichardt 1986, Medan et al. 2015), with the information traveling from the secondary eyes, to the CNS, and then to motor neurons controlling eye-tube movement (Hill 2006). This hypothesis may be supported by the fact that in iteration 2, both in the Immediate and the Delayed Circle-Bar tests, the search patterns appeared to be 'looking for' circles, suggesting that the extra 500 ms was sufficient to enact 'target searching' routines.

Alternatively, it can be suggested that although 500 ms is sufficient for priming, it requires experience for the primed stimulus (which need not be visual) to be 'recognised' and 'searched for', as demonstrated in numerous tests on humans (see Wiggs and Martin 1998). While this may account for the circular-like search patterns seen in iteration 2, but not in iteration 1, in the Immediate paradigms, it fails to explain the circular-like search patterns seen upon iteration 1 for the Delayed paradigms. Nonetheless, this latter experience-based hypothesis may have a hidden effect that is accommodated within the former computational time-based hypothesis, and only with further testing at different intervals and with different experience levels can these be accurately teased apart. While not as evident (possibly due to the size of the circle stimulus compared to the bar stimulus), these same differences exist in the Bar-Circle paradigms, strengthening the impact of these findings.

The discrepancies between the Immediate and the Delayed sessions also provide us with a glimpse into the possible neural pathways of the PL eyes, and suggest two separate afferent pathways. One extremely fast pathway results in the characteristic optomotor orientation response (Land 1971, 1972). This pathway conveys information regarding movement (or, in this case, the sudden appearance) of an object in the visual field of the PL eyes. Land (1971) suggested that this characteristic orientation response is part of an 'open loop' control system, meaning that the changes in the stimulus that occur during the response do not alter the response. A saccadic motion (being either retinal or body saccade) would result in the entire field of view of the spider to rotate. Accordingly, any static stimulus would conform to the saccade and move in the opposite direction of the saccade. Much like in Land's (1971, 1972) experiments, in this study the saccadic motions and orientation responses were also performed in the absence of corroborating visual feedback, as the spiders were tethered in place and body saccades resulted in the

polystyrene ball rapidly rotating, rather than the spider and any stimuli within its visual field. As such, the eye-tracker findings seem to substantiate and expand upon Land's (1971) notion of open loop system. All saccadic motions visible in the tracks featured a back and forth motion, initially towards the peripheral edges of the visual filed and then back, more or less to the starting point.

A likely explanation for the back and forth retinal motions would be that the first part of the saccadic motion brings the AM eye retinae to the location where the peripheral stimulus enters their visual field (through the rotation of the spider) and the second part of the retinal motion is akin to a vestibulo-ocular reflex (Fetter 2007), with the retinal motions corresponding to continual rotational motion. While beyond the scope of this study, combining eye-tracker data with recordings of the rotation of the polystyrene ball as moved by the test spider would corroborate this hypothesis. Nevertheless, the saccadic motions we see here appear to be of different lengths (i.e., the overall lengths of the red tracks), most likely a result of different saccadic speeds, strengthening this hypothesis. In accordance with the different saccadic lengths observed, Land (1971) found large variation in the rotational speed (from 164 to 1120°/s in just one animal) which showed no obvious relation to the magnitude of the rotation being executed. Taken together, a better categorisation of the salticid orientation control system would be 'partially open', as the AM eyes do respond at some level to the proprioceptive sense of movement by the spider itself. At first glance, these retinal movements might seem redundant, as visual scanning motions only begin when the retinae return to the original location. However, saccadic motions during head movements are quite common in nature (Collewijn 1977, Pratt 1982, Wallman and Letelier 1993, Dawkins 2002) and seem to primarily serve to maximise the duration of retinal image stabilisation (Wallman et. al. 1993; Land 1999).

The second afferent pathway seems to be a slower one which ultimately provides information to the efferent pathways controlling retinal movements of the AM eyes. My analysis suggests that this pathway contains not only information regarding the existence of the object, but also some form of object identification. This is apparent by the fact that expectancy violation tests typically caused an increase in the retinal search patterns and that these, at least initially, appeared to adhere to the primed shape structure rather than that presented. In contrast, the expectancy confirmation tests generally caused a decrease, or narrowing, of the search patterns. This is corroborated by the analysis of active frames, where expectancy violation resulted in an increase of overall retinal activity, while expectancy confirmation had no such effect, suggesting that the animal in the latter situation required no additional visual information. Additionally, while indirect, it is worth noting that this is the first evidence of shape recognition by the PL eyes.

This is not the first study to use expectancy-violation methods to study the effects of priming on salticids. Using predatory behaviour as an assay and lures of a dead prey item as a primer, and either the same prey in a different orientation or a different prey item as the stimulus, Cross and Jackson (2014) found that visual priming for an indeterminate period (based on how long it took the spider to face the lure for 30 s) had no significant effects on *Portia africana*'s attack latencies, but did have significant negative effects on the attack probabilities when, after being 'hidden' for 90 s, the stimulus was the alternative prey item. No such difference was found when only the orientation of the stimulus was changed. Although there are compelling similarities to my results, Cross and Jackson (2014) used behavioural assays and information available to all pairs of eyes and the 500 ms priming time used in this study can not be directly compared with their much longer and variable ones. However, this work does provide insight into Cross and Jackson's (2014) results by showing that priming affects the assessment of a stimulus, and may cause the spiders to reassess what they see.

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Chapter 6

Out of its depth: A reassessment of salticid visual depth judgements

Typical habitat of Marpissa marina (Photo: D. Harland)

Abstract

The investigation of depth perception in jumping spiders (Salticidae) has been an on-going challenge since the 1960s. The exceptional visual capability of salticids is the result of multiple camera eyes, two pairs of which are forward-facing: the anterior lateral have overlapping fields of view, while the large anterior medial or 'principal' eyes have a narrow field of view but with high spatial acuity and movable retinae. This combination gives the potential for utilization of both binocular and/or monocular depth cues. While several different processes for salticid depth perception have been suggested, evidence for these has thus far been inconclusive. Running three experiments based on jump accuracy centred on vision and on the optics of the primary eyes, this study focused on three of these processes: stereopsis – depth perception through binocular disparity; depth perception from image defocus (a mechanism based on how much the retinal image is defocused in different layers of the retina using limited wavelength information); and accommodation – depth perception by changing the optical power of the eye to maintain focus on an object as its distance varies.

By assessing jump accuracy while selectively covering different eyes, we found that even though salticids have the ability to use stereopsis, they do not use this mechanism for depth judgments. Rather, the use of monocular information from at least one of their primary eyes is both necessary and sufficient. In experiments on jump accuracy under different illumination wavelengths, no support was found for the use of image defocus for depth perception. Finally, we found distinct retinal movements that correlated with stimulus distance, which suggests the use of visual accommodation for depth judgements.

Introduction

One of the major tasks of any visual system is to convert a two-dimensional retinal image into a three-dimensional perceptual concept that is biologically informative (Marr 1982). The ability to perceive the world in a three-dimensional manner and assess the distance of an object (i.e., make depth judgements) is thus a fundamental trait. In concordance with the multitude of visual systems that have evolved (Land and Fernald 1992, Land and Nilsson 2012), animals may adopt one or more of several solutions to the problem of depth perception.

One solution adopted by many animals, including humans, is the use of stereoscopic cues (or stereopsis) for obtaining depth information. Stereopsis capitalises on the fact that different eyes have different points of view, or vantage points. By comparing the retinal disparity between two eyes (termed binocular disparity), an animal can judge the distance to the object in question (Cumming and DeAngelis 2001), as long as the eyes are not too closely spaced together. However, the further an object from the viewer, the less disparity is available for effective stereopsis. Because of their small size, arthropod eyes are typically spaced quite close together, meaning that the precision with which they can judge distance by this mechanism of triangulation is limited to nearby objects within a few centimetres (Kral 2003). For many arthropods, such as among jumping spiders (Salticidae), these distances are relevant for many biological interactions (e.g., prey capture and courtship, Richman and Jackson 1992; Jackson and Pollard 1997). While stereopsis is common and thoroughly studied among vertebrates (e.g., primates, (Cumming and DeAngelis 2001, Read and Allenmark 2013); horses, (Timney and Keil 1999); raptors, (Fox et al. 1977, Willigen 2011); toads, (Collett 1977)), it has been definitively demonstrated in only one invertebrate – the praying mantis (Rossel 1983, 1996, Nityananda et al. 2016).

For some animals, stereopsis is impossible due to the different eyes having very small or no overlapping fields of view. Consequently, one or more of several different forms of monocular depth cues are used. Common monocular cues include accommodation, or the ability of the eye to change its focus from distant to nearby objects, and motion parallax, in which the amount of image motion on the retina of a moving animal (such as one moving its head side to side) provides relative distance information because the velocity of the retinal image varies inversely with object distance (this is found in many insects; most famously in mantises and locusts; Collett 1978; Kral and Poteser 1997; Poteser and Kral 1995; Poteser et al. 1998). If information about the direction and velocity of movement is known by the animal due to proprioceptive input (information related to the animals' own position or movement), motion parallax can provide absolute depth information. Other relative depth judgements include 'height-in-plane', where distant objects appear higher in the visual scene than those nearby, as suggested for fiddler crabs (Zeil and

Hemmi 2006) and tiger beetles (Layne et al. 2006), and occlusion, in which nearby objects block objects that are farther away (Livingstone and Hubel 1988). A less-known mechanism is 'image defocus', where depth is judged by comparing how out-of-focus ('image defocus') two or more images are with respect to each other (Chaudhuri and Rajagopalan 2012, Nagata et al. 2012, Chung and Marshell 2014). Of these, only accommodation, motion parallax and image defocus are capable of providing absolute, as opposed to relative, depth perception, which is critical for visual hunters that pounce on, or strike at, their prey and must accurately assess prey distance.

Accommodation is the process by which the eye changes optical power to maintain focus on an object as its distance varies. Mammals, birds and reptiles adjust optical power by changing the form of the elastic cornea using ciliary muscles, while fish and amphibians adjust optical power by changing the distance between a rigid cornea and the retina (Harkness 1977; Kawasaki et al. 1988; Wagner and Schaeffel 1991; Ott 2006). Chameleons are unique in being the only vertebrate to focus monocularly (and independently for each eye) by using corneal accommodation for depth perception (Harkness 1977, Srinivasan 1999).

Salticidae are known for their exceptional vision, which is used to locate, stalk and finally pounce on their prey (Jackson and Pollard 1996). The final step in this process evidently requires accurate depth perception. This characteristic hunting behaviour is enabled by a visual system comprised of four pairs of eyes: two pairs of forward-facing eyes (the anterior median (AM) and anterior lateral (AL) eyes), the lateral-facing posterior median (PM; reduced in most species), and the rear-facing posterior lateral (PL) eyes (Land 1985). The latter three pairs of eyes (AL, PM, PL) are collectively known as 'secondary eyes' and each pair possesses a wide field of view, which jointly encompass c. 360^{o}, with considerable binocular overlap in the fields of view of the AL eyes (Land 1971, 1972; Zurek et al. 2010; Zurek and Nelson 2012).

The anatomically distinct AM eyes, known as 'principal eyes', provide outstanding spatial acuity (as low as 0.04°) over a narrow field of view of 3-5° (Land 1969a; Williams and McIntyre 1980; Blest et al. 1990). In his ground-breaking work using an ophthalmoscope, (Land 1969a) discovered that the retinae of the AM eyes, which lie at the end of a long innervated 'eye tube', are capable of four types of complex movements that can scan up to c. 28° from the central body axis. These movements not only compensate for the narrow field of view of the AM eyes, but are responsible for the detailed analysis of the spatial features of a visual scene.

The AM eyes are the only eyes in the salticid visual system that convey colour vision. A small number of electrophysiological studies have determined the spectral sensitivities of the AM eyes of some salticid species (reviewed in (Yamashita 1985, Nagata et al. 2012), all of which respond to green and

ultraviolet light, and possibly other wavelengths. However, data are sparse and subject to debate, with only three studies producing data from identified photoreceptors (Blest et al. 1981; Nagata et al. 2012; Zurek et al. 2015).

A curious anatomical characteristic of the AM eyes is the layered structure of their retinae (Land 1969). The receptors are arranged in four tiers, each of which lies on a different focal plane. Because of chromatic aberration due to differential refraction of wavelengths when light enters the cornea, different wavelengths are focused on different layers of the retina. This suggests that the tiered retina has a function related to colour perception, especially if each layer contains receptors of different spectral sensitivity. However, this does not appear to be the case (Devoe 1975; Blest et al. 1981; Nagata et al. 2012). Rather, in salticids with known sensitivities, the two proximal tiers (layers 1 & 2) consist of a dense, regular mosaic of green-sensitive photoreceptors, while the two distal tiers (layers 3 & 4) have a less organized mosaic structure, are UV-sensitive and also have some green-sensitive photoreceptors (Blest et al. 1981; Nagata et al. 2012). As green light is solely focused on the deepest layer (layer 1), but receptors sensitive to green light are found in the more distal layers, speculation arises regarding the purpose of this arrangement (Land 1969a; Blest et al. 1981; Harland and Jackson 2000). Recently, Nagata et al. (2012) suggested that this structure enables depth perception. In theory, by comparing the level of defocus of the same image in two different layers of the retina, salticids could assess the absolute distance of an object. Nagata et al. (2012) tested salticid pounce accuracy on an unrestrained live fly under either green or red light. As the focal length of red light is greater than that of green light, the defocus amount under red light would be equal to that generated by a closer object under green light. Thus, under red light, spiders were expected to undershoot the target, which was what Nagata et al. (2012) found. Their findings were largely based on elegant molecular and electrophysiological work; however, the sample size for the behavioural experiments was four, somewhat weakening the basis of their work. Additionally, while molecular and electrophysiological data suggest that most salticids seem unable to see red light (Devoe 1975; Blest et al. 1981; Nagata et al. 2012), Zurek et al. (2015) demonstrated that at least one species, *Habronattus pyrrithrix*, has long-pass spectral filters in layer 1, shifting the sensitivity of a subset of their photoreceptors from green to red.

Salticid secondary eyes are fundamentally different from the AM eyes. They do not achieve the same spatial acuity as the AM eyes, and their retinae are immobile (Homann 1928). Their main function is the acute detection of movement, upon which the spider turns to face the source of this movement with the AM eyes (Zurek et al. 2010; Zurek and Nelson 2012). Of the two forward-facing pairs of eyes (AL and AM), the AL eyes have a larger and also overlapping field of view (from c. -25° to +60° horizontally), with a region

of binocular overlap encompassing roughly 30°, and overlapping the frontal zone of the field of view of the AM eyes (Figure 1). Moreover, the retinae of the AL eyes contain an acute zone which, while not as sharp as the AM eyes, supports higher resolution than those of keen-sighted insects (Land 1985a,b). These two characteristics suggest the use of stereopsis for depth perception, yet to date this has not been rigorously studied.

In this study, three approaches have been used to investigate the mechanisms of short to medium distance (up to 25 cm) depth perception in salticids. Initially, we tested whether spiders use binocular and/or monocular cues for depth perception. We then attempted to replicate the behavioural findings from Nagata et al. (2012), testing depth perception through image defocus using two salticid species that differ significantly in their natural light environments. Finally, using an 'eye-tracker' (see Appendix 2), we filmed the retinal movements of the AM eyes when viewing a stimulus at different distances, with the objective of identifying whether AM eye retinal movements correlate with object distance, as this could be indicative of an 'accommodation' type of process.

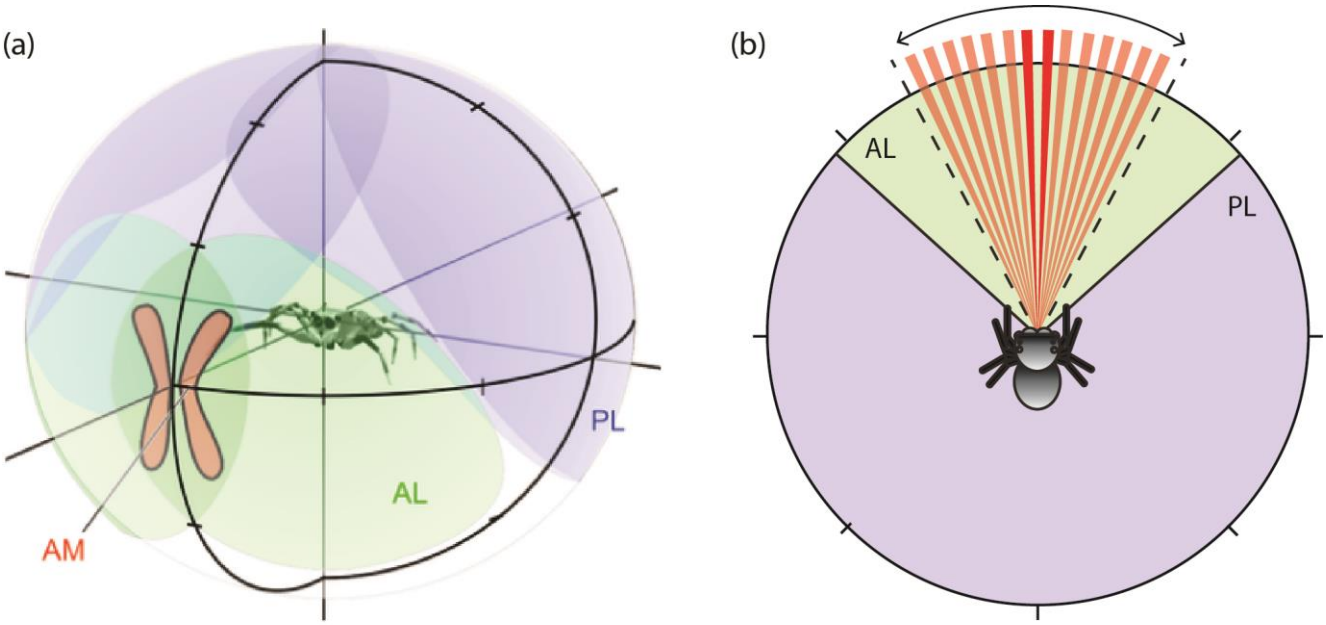

Figure 1: Typical fields of view of the three main eye pairs of a salticid spider (Anterior median or AM (red), anterior lateral or AL (green), posterior lateral or PL (purple)). (A) Boomerang-shaped AM or primary eye retinae fields of view lie within the overlapping fields of view of the AL eyes. (B) Dashed lines indicate range of movement of the AM eye tubes. (A) Reprinted with permission from Duane Harland; (B) redrawn with permission from Daniel Zurek.

Methods

(a) *General*

The two species used in these studies were *Servaea incana* and *Marpissa marina*. *Servaea incana* is a large salticid (c. 8-10 mm) common in temperate eastern Australia, where it lives under loose bark on *Eucalyptus* trees, hunting insects on and under the bark (Richardson and Gunter 2012, McGinley et al. 2016), in a high-contrast and often very dark environment. *Marpissa marina* is a similar-sized salticid native to the South island of New Zealand, where it lives on rocky shores, 2-6 m above the high tide mark, hunting in an extremely bright and unshaded shoreline environment (Vink 2015, pers. obs.).

All spiders were kept in a temperature-controlled laboratory set to 24^{o}C - 26^{o}C, with a photoperiod of 12L:12D (lights on at 07:00). Spiders were housed individually in 1 litre cylindrical transparent plastic jars, with a cotton roll inserted into a hole made into in the bottom of the jar and extending into a cup of water that was topped-up as needed, providing humidity. The cages had two additional holes of 1 cm diameter at the top, one covered with cotton gauze for ventilation and another 'feeding hole', which was plugged with a cork. Cages were enriched with sticks that were inserted through folded card within which the spiders could build nests or hide. All spiders were fed 1-3 house flies (*Musca domestica*) and a variety of field caught dipterans (mass equivalent to c. 1 house fly) weekly. For consistency, test spiders were unmated adult males and females that were starved for 5-7 days prior to testing. All tests were carried out between 08:00 and 14:00. For all experiments, statistical analyses were performed using SPSS v20, GraphPad Prism v6 and R v3.01. The specific analyses used for each experiment are detailed below.

(b) *Binocular and monocular cues 'flying saucer' experiment*

For this experiment, adult male and female *Servaea* were used. The goal was to determine whether spiders use binocular cues and/or monocular cues for depth perception, and which pair of forward-facing eyes (AL or AM) was responsible. Consequently, spiders were subjected to the following treatments in randomised order: 1. a control group in which no AL or AM eyes were covered; 2. two AL eyes covered; 3. one AL eye and opposing AM eye covered; 4. one AM eye available (both AL and one AM eye covered); 5. both AM eyes covered; 6. one AL eye available (both AM and one AL eye covered) (Fig. 1). The different AM and AL eyes were occluded with silicone, and in all cases the PL and PM eyes were also covered. The control group

received a single dot of silicone placed on the cephalothorax to control for the weight of silicon in eye occlusion treatments.

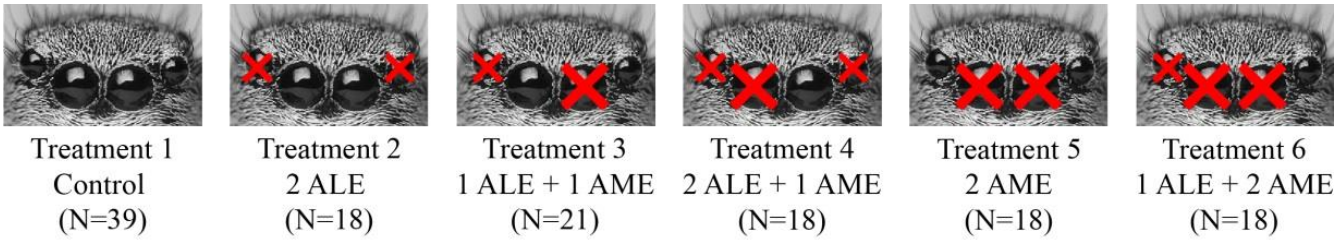

Figure 2: The different eye occlusion treatments used (and N) in the flying saucer experiment. 1. a control group in which no AL or AM eyes were covered (N = 39); 2. two AL eyes covered (N = 18); 3. one AL eye and opposing AM eye covered (N = 21); 4. one AM eye available (both AL and one AM eye covered; N = 18); 5. both AM eyes covered (N = 18); 6. one AL eye available (both AM and one AL eye covered; N = 18)

For eye-occlusion treatments, spiders were restrained within a plunger with a foam base and in which the outer tube contained holes, allowing access to the cephalothorax. Using a stereomicroscope, the eyes were covered using dental silicone (Coltene Whaledent President), which sets to become a smooth, easily removable, opaque elastomer (see Zurek et al. 2010 for details).

Tests were designed to capitalise on salticid motivation to leave an area if surrounded by water (pers. obs.). Spiders were placed on an 'island' in the centre of a 35 cm diameter circular aluminium dish and were provided with options for getting out by jumping on outer 'islands', referred to here as 'saucers'. The dish was filled with water to a height of 15 mm (the 'lake'). This water height was chosen because any reflection of the saucers on the water was refracted (by the water and by the bottom of the aluminium dish) in such a way as to create distorted images which appeared fuzzy, and thus could not be used as an accurate cue for distance or height estimation. The central island was a 55 mm tall plastic vial (25 mm wide) rimmed by green plastic sheeting (16 mm wide, c. 1 mm thick) for a total island diameter of 57 mm at a height of 40 mm from the water level. In the centre of the island was a 'pit', created by filling the open end of the vial with cotton to a depth of 10 mm from the top edge and covering the surface of the cotton with green plastic sheeting. Prior to trials starting, I placed a spider within the pit for 5 min and covered it using a glass coverslip. If spiders were reluctant to leave the pit once the coverslip was opened they were gently coaxed out by touching their legs with a paintbrush.

The central island was surrounded by an assortment of saucers, which were purple discs of plastic sheet with a central black dot made with a permanent marker pen. Saucers were of two sizes at a 1:1.7 ratio: small (10 mm diameter; central black dot 3 mm) and large (17 mm diameter, central dot 5 mm). To

minimize potential cues which could be used for depth perception, glass microcapillaries attached to the saucers were placed at a 24^{o} angle from the substrate, such that the capillaries were obscured from view in their entirety by the saucer itself, which would therefore appear to be 'floating' on air (Figure 3); thus giving rise to their moniker 'saucer', as they appeared like flying saucers. Saucers were placed in alternating manner at 45^{o} from each other in a circle around the central island, such that eight saucers (four of each size) surrounded the central island. The distance and height of the saucers from the central island were always consistent for each saucer size, such that they differed on a 1:1.7 ratio. Large saucers were placed 50 mm from the edge of the island to the centre of the saucer (42.5 mm from island edge to saucer edge) at a height of 38 mm above the substrate, and small saucers were placed 30 mm from the edge of the island to the centre of the saucer (25 mm from edge to edge) at a height of 45 mm. These distances and heights ensured that the visual angle subtended at salticid eye height from the rim of the central island (after leaving the pit spiders always spent their time on the rim 'looking' for an exit and prior to jumping) was identical for both saucer sizes.

Under natural conditions, a visual scene is composed of objects located at different depths, yielding different patterns of retinal motion. This scenario was prevented by surrounding the entire apparatus by a 1 m high cylinder of white card. Plastic saucers, the central island, and the coverslip were wiped with ethanol after all tests to remove chemical traces of previous tests.

Spiders were given 5 attempts to jump accurately on a saucer before tests were terminated (scored as 5 attempts). If spiders landed in the water they were gently picked up with a paintbrush, placed back in the pit and covered until the spider became quiescent. Salticids which were unresponsive on the rim of the island for 5 min were scored as a 'failed' jump attempt and the spider was placed back into the pit (for a maximum of 5 attempts). All trials were filmed from above using a Canon PowerShot G16 at 30 frames per second for subsequent scoring.

Friedman tests and Wilcoxon Signed Ranks tests were used to test for differences in total test duration, number of attempted jumps, number and proportion of 'directed' jumps (i.e., jumps that were directed to within 10^{o} of a saucer), and successful jumps (i.e., jumps which ended with the spider landing on a saucer) for both saucer sizes combined and separately. Binomial tests were used to compare the distribution of saucer sizes targeted by the spiders within the different treatments, and Fisher-exact tests were used to compare the success rates per saucer size within each treatment.

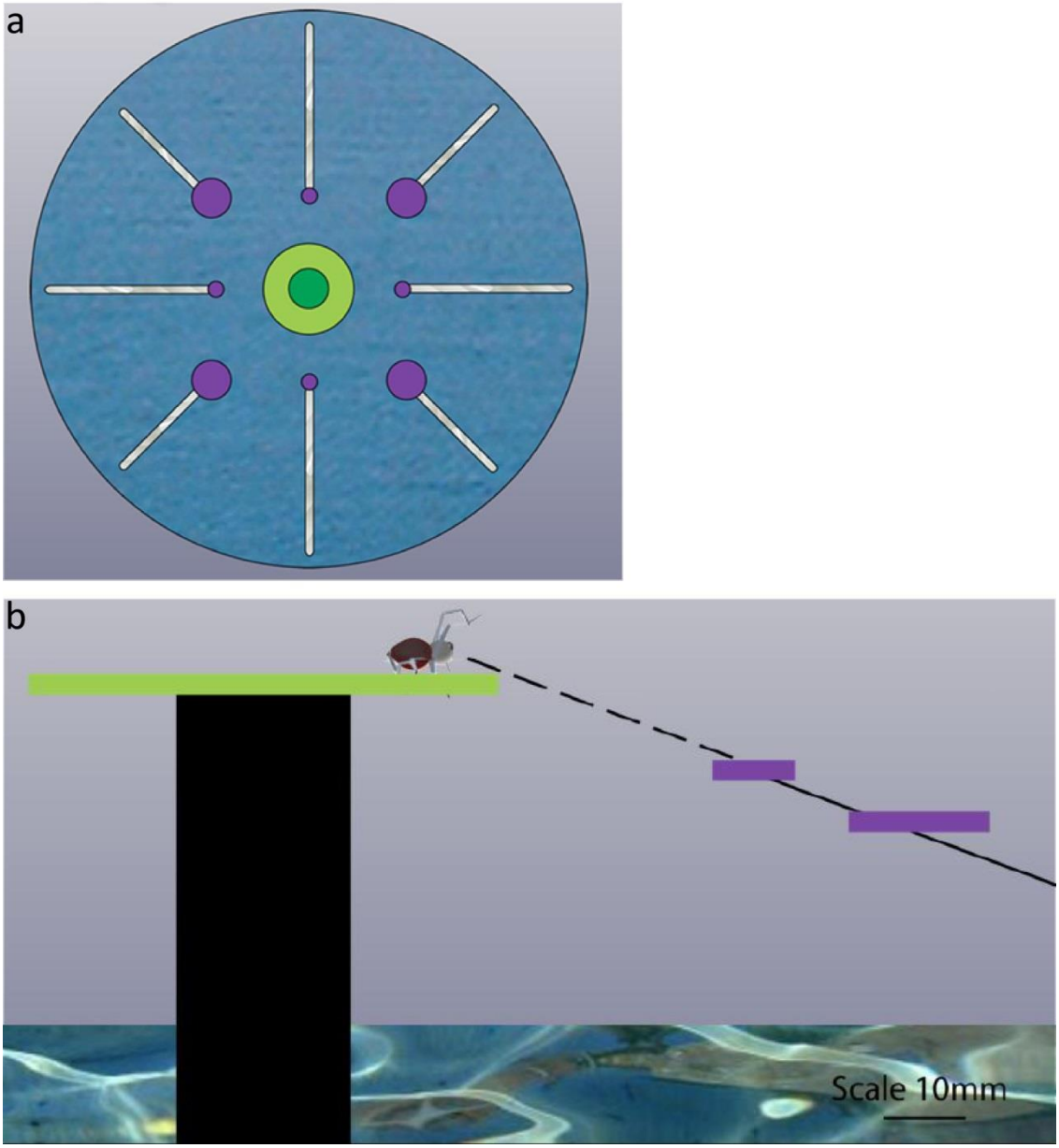

Figure 3: Layout of the flying saucer experiment. (a) Aerial view. Salticids were placed on the central island (green) which was surrounded by water and eight purple saucers (four large, four small) held in place with angled glass capillaries. (b) Cross section of the saucer experiment. Large and small saucers subtended the same visual angle from the point of view of the spider due to their distance.

(c) *Depth perception under different wavelengths*

Nagata et al. (2012) tested the use of image defocus for depth perception, with the expectation that spiders, when hunting, would undershoot their pounce on an unrestrained fly under red light. The goal of my experiments was to try and corroborate these findings using a larger sample size, stimuli which were restrained (but able to move their wings, thus limiting uncontrolled effects of prey locomotion on pounce accuracy) and using two salticid species differing in the light habitats in which they naturally live. The latter allowed us to consider whether adaptation to distinct light environments led to differences in the use of different wavelengths.

Wild-caught mature spiders of both *Marpissa* and *Servaea* were used within two months of capture. Experiments were performed in plastic square arena (25 X 25 X 7 cm) sitting on top of a laminated 5 mm paper grid. The arena was covered with glass to prevent the spiders from walking out and was uniformly illuminated by 6 light-emitting diodes placed in a 12 cm ring, 60 cm above the centre of the arena. LEDs were specified as peaking at 520 nm and 640 nm, and were also measured using Ocean Optics USB2000+ and SpectraSuite. Additionally, we measured LED reflected intensity from the arena substrate at 20 W/m2 and 18 W/m2, for green and red, respectively.

The stimulus used was a live *Drosophila melanogaster* glued to a glass cover slip by dipping its legs into a drop of beeswax. The stimulus was placed in the centre of the arena just under the light ring. Spiders were placed into the arena at random locations at least 10 body lengths away from the fly. Sessions ended when the spider pounced on the prey (either hitting or missing), or when 20 min had elapsed. The sessions were recorded from above with a DV camera (Canon PowerShot G16) at 60 frames per second. Pounces and attempted pounces were analysed frame-by-frame using ImageJ (1.49j10) and Adobe Photoshop CS6. Pounce distance was used as a proxy for the distance estimated by the spiders. Using t-tests, we compared differences in pouncing distance between different wavelengths and between cases when the spider hit or missed the prey. Fisher exact tests were used to analyse the propensity of the spiders to attempt a pounce under different wavelengths.

(d) *Involvement of retinal movement in depth perception*

In this experiment we attempted to identify whether AM eye retinal movements correlate with object distance, which could be interpreted as the use of ‘accommodation’ for depth perception via retinal movements. To do this an eye-tracker was used to record the salticid retinal movements while a stimulus was moved nearer and further from the spider.

The eye-tracker (for details see Appendix 2) is an ophthalmoscope (a tool for viewing retinae) which has been modified by adding two extra ports – an input port where a stimulus can be presented (while still enabling a view of the retinae), and an output port with an IR video camera for live viewing and recording of the retinae and their movements. As stimuli, we typically use digital images back-projected onto a white screen through a lens placed 10 mm from a projector (AAXA M2 Micro Projector), with the screen and lens being held in place using a 3D printed 'spider theatre' attached to the eye-tracker (Figure 4). For this experiment we modified the eye-tracker by introducing a movable stimulus (Figure 4; Appendix 3, Figure S1). This was done by disconnecting the 'spider theatre' from the eye-tracker and placing it, with the projector, on a moving platform that allowed 10 mm of forward and backward movement. Under the optics of the eye-tracker this translates to apparent image distances of 70 mm and 260 mm from the point of view of the salticid. As a stimulus we used a digital stick figure image of a jumping spider used in previous experiments (Figure 4). This stimulus was selected to encourage attention of the spider being tested, while keeping the stimulus simple.

Test spiders were adult female *Servaea* (N = 3). Spiders were tethered in place using dental microapplicators dabbed in bees wax and then attached to the spiders' cephalothorax, which was then placed in a micromanipulator, allowing fine positioning of the spider in front of the eye-tracker. Once a clear image of the spider retinae was obtained, the test session began by presenting the stimulus. Each session consisted of 100 s of video recording of the retinal movements while the stimulus platform was moved back and forth every 10 s. During each session, whether the stimulus was far or near was encoded within the output video by the presence or absence of a dot (trial signal).

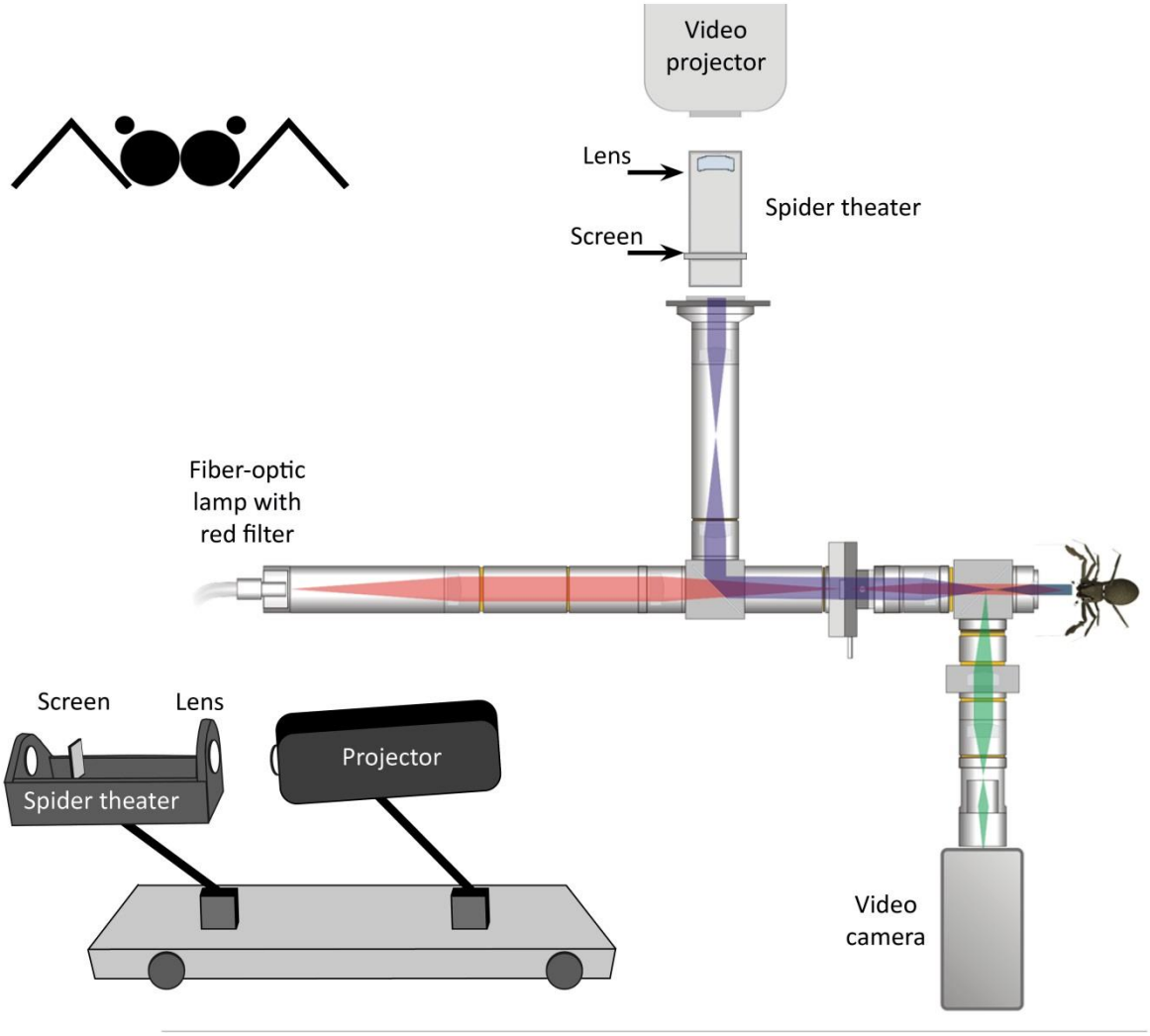

Figure 4: Eye-tracker layout depicting the different light pathways. Near infra-red light (red) is projected into the spider's eyes and reflected (green) into the video camera. A stimulus is projected (purple) through a reducing lens onto a screen which is visible to the spider. Top inset: stick figure stimulus used in experiments, depicting a jumping spider as viewed from the front. Bottom inset: spider theatre and projector on-top of the moving platform.

Spiders were set up in order to get the clearest possible image of one retina to enable reproducible precision to measurements. This meant that one retina was less clear. All spiders were arranged so that the left retina was used for measurements. Prior to analysis, each 100 s video was split into individual frames (3000 frames) for measurement and the individual frames were contrast enhanced. Frames where the retina did not move (i.e., identical frames) were identified and removed using ImageJ. Of the resulting frames, only those where the left retina was within a predefined location were selected. This location was chosen in such a way as to maximise the count and accuracy of retinal measurements. This ensured that

any differences in retinal measurements represented an actual change, rather than one resulting from the optics of the eye-tracker.

Blind scoring was done by randomly reordering the resulting frames of all videos and removing the trial signal information (whether the stimulus was near or far) from each frame. Frames were analysed by two separate blind scorers. We measured the horizontal angles of the outer lines of the 'dorsal arm' and 'ventral arm', as well as the outer and inner angle between the arms (Figure 5).

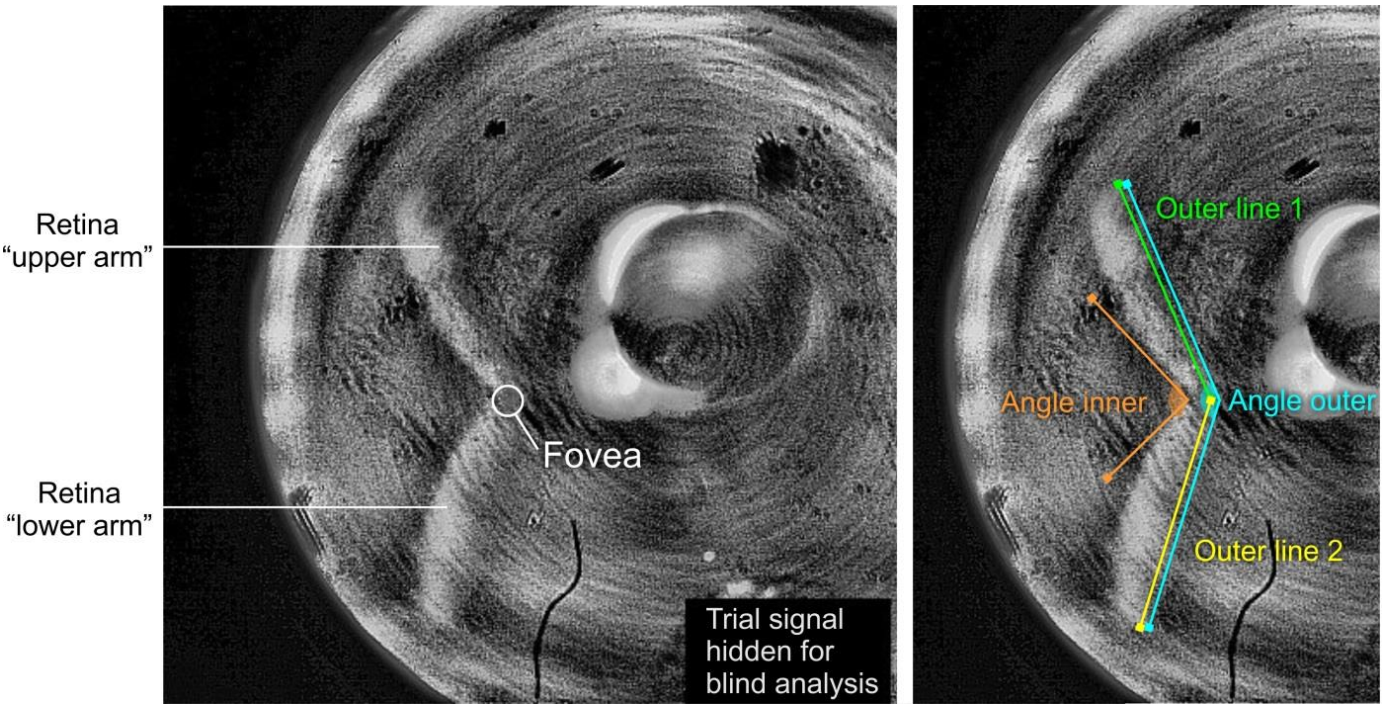

Figure 5: Eye-tracker images of salticid AM retinae. Left: Image of the left retina, with fovea depicted in the middle, as seen after image enhancement. Right: the four angular measurements taken for each frame. Redrawn with permission from Duane Harland.

Prior to analysis, cross correlations were performed on the measurements of each scorer to verify measurement soundness (inter observer reliability, r = 86). Analysis was done for each spider independently and Wilcoxon signed-rank tests were used to compare the retinal measurements of frames corresponding to a near stimulus with those corresponding to a far stimulus.

Results

(a) *Binocular and monocular cues in flying saucer experiment*

Treatment (see Figure 2) had a strong effect on the total test duration (χ^2 = 46.143, df = 5, p < 0.001, Figure 6a) and the total number of attempted jumps (χ^2 = 61.202, df = 5, p < 0.001, Figure 6B). For both measurements, Bonferroni-corrected analyses between pairs of treatments that had the same number of AM eyes covered (i.e., treatments 1 and 2; 3 and 4; 5 and 6; showed no significant differences (Figures 6a,b). In contrast, other than when comparing between treatment 3 (one AL eye and opposing AM eye covered) vs treatment 6 (both AM and one AL eye covered) (Figure 2), there were significant differences (in all cases, p < 0.001) in all pairwise comparisons between treatments that differed in the number of AM eyes covered (Figures 6a,b). As there were not enough directed jumps (jumps directed within 10^o of a saucer) performed under treatment 5 (both AM eyes covered), this treatment was removed from all further analyses (2 directed from 10 attempted jumps). Treatment had a strong effect on the number of directed jumps (χ^2 = 64.824, df = 5, p < 0.001, Figure 6c), and a non-significant trend on the proportion of directed jumps from the total number of jumps attempted (χ^2 = 8.220, df = 4, p = 0.084, Figure 6d). No difference in success rates of directed jumps to either saucer size was found between the control and treatments 2-4 (control vs treatments 2 [two AL eyes covered], 3, and 4 [both AL and one AM eye covered]; respectively: Z = -2.1, p = 0.36; Z = -1, p = 0.317; Z = -1.323, p = 0.186; Figure 6e). However, under treatment 6 the spiders were significantly less likely to land on a saucer than in the control (Z = -3.187, p < 0.005, Figure 6e) with only 2 of 31 directed jumps (6.5%) landing on a saucer in treatment 6, and 95 of 191 (50%) in the control.

When comparing the distribution of saucers targeted by the spiders within each treatment, I found that the spiders were equally likely to direct their jumps to small or large saucers (small/large: control, 102/89, p = 0.385; treatment 2, 36/71, p = 1; treatment 3, 71/127, p = 0.214; treatment 4, 72/52, p = 0.080; treatment 6, 16/15, p = 1; Figure 5f). However, in the control group, as well as in treatments 2 and 3, test spiders were significantly more likely to successfully land on the smaller, nearer, saucer than on the larger one (p < 0.001; p < 0.001; p < 0.005; respectively) with a non-significant trend for this in treatment 4 (p = 0.067). This analysis was not performed for treatment 6 as there were only two successful jumps.

Finally, when comparing the success rates per saucer size between the different treatments (control and treatments 2-4 only, Figure 2), no significant differences were found for either saucer size (small: χ^2 = 6.143, df = 3, p = 0.185; large: χ^2 = 2.786, df = 3, p = 0.426; Friedman test). Again, these analyses were done without treatments 5 and 6 as there were not enough jumps to analyse.

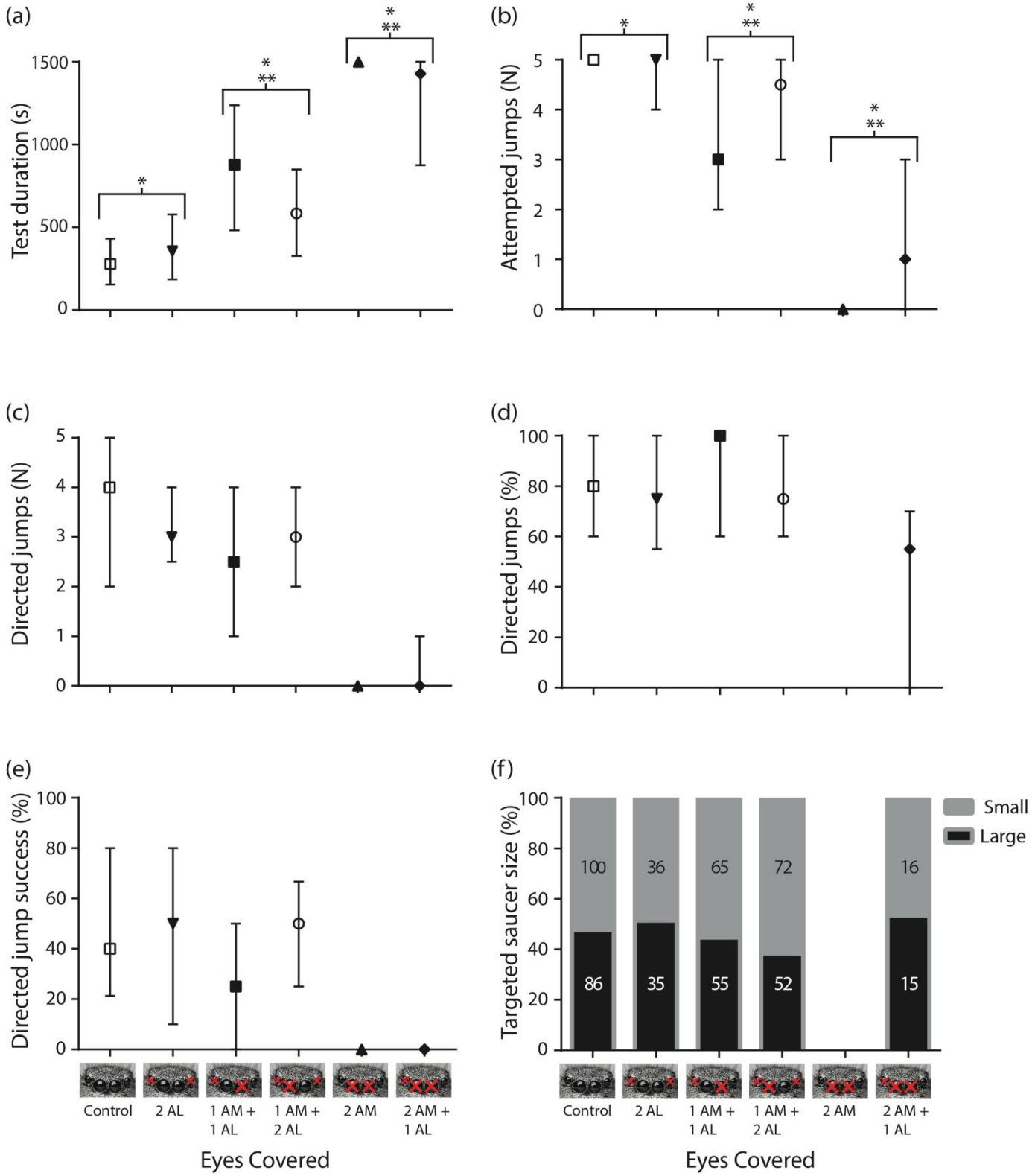

Figure 6: Results from the binocular and monocular cues experiments. Panels (**a-e**) depict median results with interquartile ranges. Panel (**f**) shows the percentages of all targeted saucer sizes under each treatment, with N for each group within the bar. *Pairwise analyses showed no significant differences within groups, but significant differences ($p < 0.001$) between them, except for (**) treatments 3 (one AL eye and opposing AM eye covered) and 6 (both AM and one AL eye covered).

(b) *Depth perception under different wavelengths*

The likelihood of pouncing on the prey was significantly higher under the green light than under the red light in both species. *Marpissa* attempted to pounce on the prey 90% of the time under the green light and 59% of the time under the red light (pounced/total tested: 37/41 and 26/44, respectively; $p < 0.005$). *Servaea* attempted to pounce on the prey 63% of the time under the green light and 34% of the time under the red light (pounced/total tested: 27/43 and 20/59, respectively; $p < 0.01$).

For both species tested, success (i.e., the likelihood of landing on the prey on the first pounce attempt) was not significantly different under green or red light. *Marpissa*'s *s*uccess was 73% under green light and 69% under red light (hit/miss: 27/10 and 18/8, respectively; $p = 0.783$). *Servaea*'s *s*uccess was 85% under green light and 70% under red light (hit/miss: 23/4 and 14/6, respectively; $p = 0.286$).

In the cases when the spiders missed the prey, there was no significant difference, for either species, in the distance by which the spiders missed the prey (i.e., overshooting or undershooting) under green or red light - although for *Marpissa* this was close to significant, with the missed distance under the red light being farther than the green light by about three mm (Table 1, Figure 7). Additionally, no significant differences were found when comparing the pounce distance (i.e., the distance from the prey when the spider attempted to pounce on it) between the different wavelengths (Table 2), nor when comparing the instances when the spiders hit or missed the prey under each wavelength (Table 3).

When comparing between the different species, there were no significant differences in success for either wavelength (green light: $p = 0.360$; red light: $p = 1$), however, there were significant differences in the number of attempted jumps under both wavelengths (green light: $p < 0.005$; red light: $p < 0.05$), with *Marpissa* attempting more jumps in both cases.

Table 1: Mean distances (and N) by which the spiders missed prey at different wavelengths. T-tests were performed to check for differences in pounce distance under the different wavelengths for the different species.

Species	Wavelength	N	Mean missed distance (mm)	Std. deviation	t	df	p
Marpissa marina	Green	10	-1.57	2.96	2.039	16	0.058
	Red	8	2.23	4.9			
Servaea incana	Green	4	0.05	6.85	-0.78	8	0.458
	Red	6	-2.63	4.13			

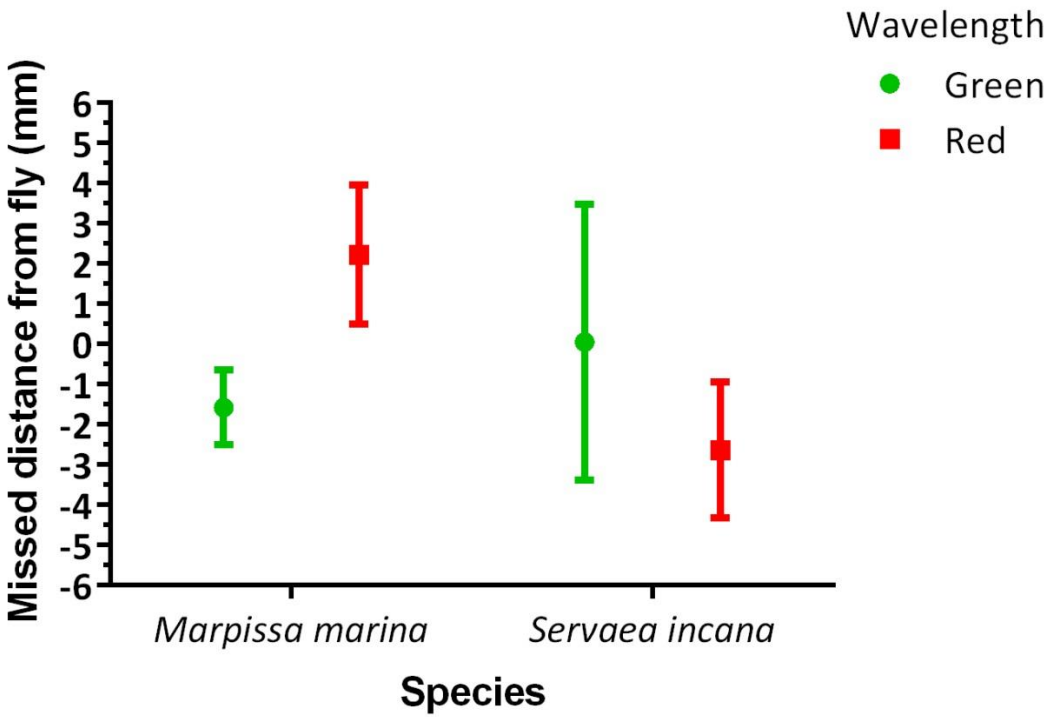

Figure 7: Mean (and SEM) distribution of distances by which pouncing spiders undershot or overshot prey under different wavelengths.

Table 2: Mean distances (and N) from which the spiders attempted a pounce (regardless of success) under different wavelengths. T-tests were performed to check for differences in pounce distance under the different wavelengths for the different species.

Species	Wavelength	N	Mean pounce distance (mm)	Std. Deviation	t	df	p
Marpissa marina	Green	37	9.51	6.58	0.846	61	0.401
	Red	26	11.05	7.83			
Servaea incana	Green	27	6.99	3.35	-0.068	45	0.946
	Red	20	6.93	2.97			

Table 3: Descriptive statistics of distances from which spiders pounced under different wavelengths. T-tests were performed to check for differences in pounce distance under the different wavelengths for the different species.

Species	Wavelength	Missed	N	Minimum (mm)	Maximum (mm)	Mean (mm)	Std. Deviation	t	df	p
Marpissa marina	Green	No	27	1.7	19.9	9.97	5.5	-0.697	35	0.490
		Yes	10	1.2	25.2	8.26	9.1			
	Red	No	18	3	25.7	12.97	8.4	-1.981	24	0.059
		Yes	8	2.4	13.1	6.73	4			
Servaea incana	Green	No	23	2	15.4	7.45	3.3	-1.785	25	0.086
		Yes	4	2	8.3	4.34	2.8			
	Red	No	14	3.5	11.9	6.95	2.7	-0.04	18	0.968
		Yes	6	2.3	12.2	6.88	3.9			

(c) *Involvement of retinal movement in depth perception*

When comparing the measurements of a single retina between instances of the spider viewing a near stimulus and a far stimulus, significant differences were found for the angles of both the dorsal and ventral retinal arms of all three spiders (Table 4). In all cases, the angles changed counter-clockwise when moving from the near stimulus to the far stimulus. While the difference in the mean Δ of angle change between the dorsal and ventral retinal arms might suggest that they move independently, this is merely an artifact of averages and non-centric torsional movement. Significant differences were also found between the inner angles in two of the three spiders, with the inner angle getting smaller when moving from the near stimulus to the far stimulus (spiders 1 and 3, Table 5, Figure 8a). In spider 2, though approaching significance (Table 5), no such differences were found in the inner angle. However, by running an outlier analysis on all data using ROUTs method with Q=1%, one outlier was identified for spider 2. Post removal, significant differences were also found between the inner angles for spider 2 (Table 5). No significant differences were found between the outer angles in any of the spiders (Table 5, Figure 8b).

Table 4: Changes in the mean angles of the dorsal and ventral arms of the salticid retina correlated to stimulus distance. All Δ values relate to anticlockwise rotation. Actual angles are not given as the horizon is not exact for each spider. Analyses are Wilcoxon signed-rank tests.

Spider	N frames (far; near)	Δ Mean dorsal arm angle	Δ Mean ventral arm angle	Dorsal arm angle (Z, p)	Ventral arm angle (Z, p)
1	69; 46	2.5°	3°	Z = -5.075, p < 0.001	Z = -5.73, p < 0.001
2	89; 85	2.8°	3.5°	Z = -3.061, p < 0.005	Z = -6.382, p < 0.001
3	78; 112	3.5°	2.8°	Z = -5.466, p < 0.001	Z = -5.038, p < 0.001

Table 5: Changes in the mean inner and outer angles of the salticid retina correlated to stimulus distance. Analyses are Wilcoxon signed-rank tests.

Spider	Inner angle (far; near)	Outer angle (far; near)	Inner angles (Z, p)	Outer angles (Z, p)
1	89.2°; 92.7°	136°; 137°	*Z* = -2.201, p < 0.05	*Z* = -1.502, p = 0.133
2	90.3°; 93.2°	136.6°; 136.1°	*Z* = -1.864 (-2.087)*, p = 0.062 (< 0.05)*	*Z* = -0.62, p = 0.535
3	92.2°; 94.5°	137.2°; 137.8°	*Z* = -2.124, p < 0.05	*Z* = -1.098, p = 0.272

*Statistics refer to before and after (in parentheses) the removal of an outlier

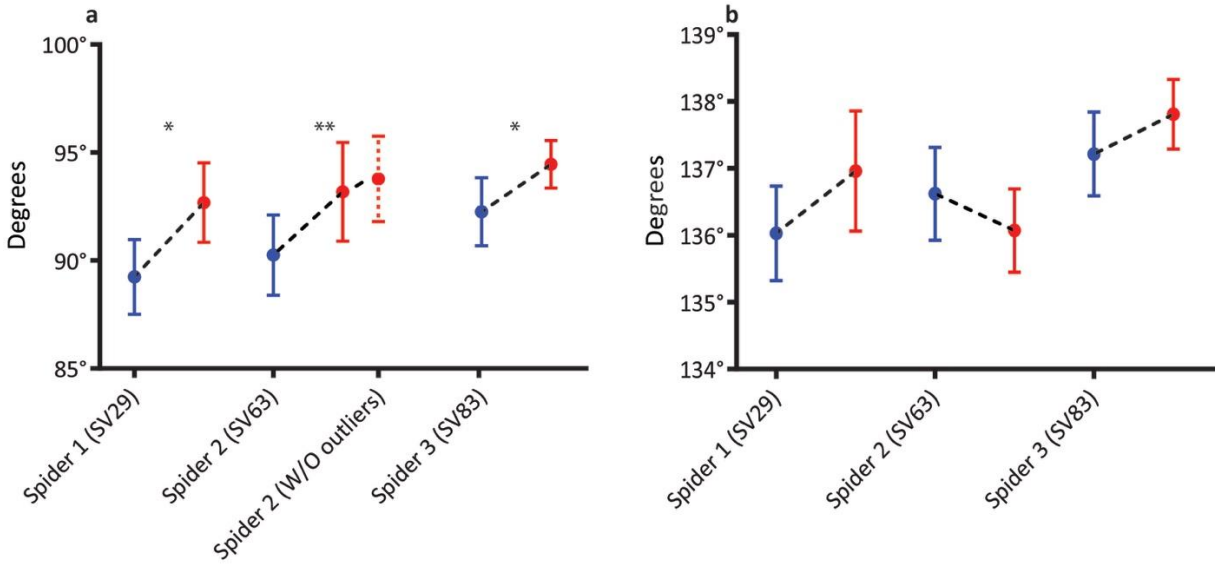

Figure 8: Differences in the mean inner (a) and outer (b) angles of the salticid principal eye retina between instances when the spiders were viewing far (blue) and near (red) stimuli. Error bars represent 95% confidence levels. *p < 0.05, **p = 0.06 before removing outlier (solid red error bar) and p < 0.05 after removing outlier in data (dashed red bar).

Discussion

(a) *Binocular and monocular cues in 'flying saucer' experiments*

The results from the flying saucer experiment strongly suggest that salticids are capable of making monocular depth assessments using a single AM eye and that the AL eyes are not necessary for depth perception to distances up to 30 body lengths away. In fact, losing the ability to see through one AL eye, which would disrupt binocular vision mediated by these eyes with large binocular overlap (Land 1985b), seems to make no obvious difference to having all frontal-facing eyes with which to make an assessment, as exemplified by the proportion of directed jumps, the proportion of successful jumps, and the distribution of targeted saucer sizes. In contrast, losing the ability to see through one AM eye has a strong effect on the spiders' motivation, as measured by test duration and the number of attempted jumps. However, there were no measurable effects on the ability of the spiders to accurately gauge depth as long as at least one AM eye was left uncovered. In the few cases that the spider did attempt a jump with both AM eyes covered, success rate was negligible.

The use of 'height-in-plane' as a monocular basis from which to make depth judgments seems unlikely, as the flying saucer experiments were designed to prevent comparative cues on the same horizon. Moreover, the distances and visual angles of the different saucer sizes were specifically set up to prevent size or occlusion cues. Though the use of relative target height cannot be ruled out as a depth cue for salticids in nature, it is unlikely to have evolved as their natural environment is typically very cluttered (rather than a 'flat-world' environment'; see Zeil and Hemmi 2006; Layne et al. 2006) and the fact that salticids regularly perform accurate jumps, regardless of their orientation relative to their target or to gravity. While distance estimation often requires both proprioceptive and exteroceptive (information related to external movement) input (Cornilleau-Pérès and Gielen 1996; Poteser et al. 1998), the situation examined here is unlikely to have involved proprioceptive input because salticids are stationary before a jump (Forster 1977).

Prior to jumping there was never any observable motion of the cephalothorax, either vertically or by translation, as might be expected for motion parallax (XJN pers. obs.). This leaves two possible monocular depth cues that could explain these data: accommodation, and the 'image defocus' hypothesis proposed by Nagata et al. (2012) in which depth perception is mediated by a comparative analysis of chromatic aberration within the tiered retinae of the AM eyes. However, neither hypothesis fully explains why it seems that having two AM eyes allows spiders to judge depth faster than when only one AM eye is available, as seen in the total test duration of the flying saucer experiment. It appears that even though salticids are capable of making accurate depth judgments monocularly, they are reluctant to do so.

The solution may be found in one of the most noteworthy aspects of these results, which is the strong and additive effects of the loss of AM eyes on the spiders' behaviour. For each eye AM eye covered, the spiders approximately doubled their test duration, while halving the total attempted jumps. In the absence of information from the AM eyes, spiders took a very long time (usually the maximum allowed) to do these tests. This is most likely the result of conflicting information from the uncovered AL eyes and the covered AM eyes. It would be interesting to investigate the plasticity of the spiders' visual system to determine whether they overcome the loss of one AM eye.

The AL eyes are evidently not necessary for depth judgments and are likely not used for stereopsis, but it was impossible to conclude if they are sufficient for depth perception (whereas at least one AM eye is both necessary and sufficient), as without the AM eyes the spiders were very reluctant to move at all. As Blest et al. (1981) stated, given the binocularity of the AL eyes, it is surprising that they cannot act as rangefinders by themselves. It is possible that a spider whose AM eyes have been blinded 'refuses' to make jumps, not because it is unable to estimate distances, but as a consequence of its inability to precisely identify what it might land on. However, this seems unlikely, as acuity in the AL eyes is comparable to that of the best compound eyes of insects that fly and accurately land on small objects (Land 1985a,b).

Why there is such a large region of binocular overlap (Land 1985b) in the fields of view of the AL eyes thus remains unanswered. This region of binocular overlap also comprises the region of highest acuity (fovea) of the AL eyes, as measured by photoreceptor density (Blest et al. 1990), suggesting that perhaps this region serves primarily to complement - in a wider 'snapshot' field of view - the highly spatially acute information provided by the AM eyes, whose moveable region exceeds the region of binocularity of the AL eye, but whose small field of view requires considerable time to sweep across a visual scene. Kral (2003) suggests that, with few exceptions, distance estimation in insects requires binocular input. Evidently, this is another way in which spiders differ from insects.

(b) *Depth perception under different wavelengths*

I was unable to replicate the results from Nagata et al. (2012), finding no measurable effect of the different wavelengths on the pouncing characteristics of either species tested. While the spiders were more likely to attempt a pounce on the prey under the green light than the red light, there was no effect of wavelength on jump accuracy, with over 70% successful attempts under both wavelengths. Despite the fact that the two species were from vastly different light environments and were tested shortly after collection from the field, jump accuracy under the different wavelengths was comparable. Moreover, in the instances in which the spiders did miss the target, they were equally likely to overshoot or undershoot the prey. On the

assumption that depth perception occurs through chromatic aberration, spiders should be undershooting prey under red light, as reported for *Hasarius adansoni* (Nagata et al. 2012). Conversely, in *Marpissa,* the trend was to overshoot the prey under the red wavelength.

These data suggest that, at least for these species of salticids, chromatic aberration (or image defocus) does not mediate depth perception. This is notable in *Marpissa*, which, even though they live in a bright environment with ample light in which to make comparisons, do not seem to have evolved the use of this type of information as a depth perception cue. Nevertheless, in these experiments, unlike the ones in Nagata et al. 2012, the AL eyes were left uncovered. However, rather than causing negative effects, this difference should have increased the accuracy.

(c) *Involvement of retinal movement in depth perception*

Two distinct types of retinal motion correlated to stimulus distance were observed when the stimulus was moved from the closer to the further position. Firstly, a counter-clockwise torsional motion of about 3^{o} (measured by the out angles of the dorsal and ventral retinal arms), and secondly, a closing of the inner angle of the retina by about 3^{o} (figure 9). The torsional motion that was detected in this experiment was first observed by Land (1969b), who noted that horizontal and torsional movements can occur simultaneously and regarded them as part of the four scanning motions that he described. Blest et al. (1981) later suggested that these motions, as well as the typical back and forth scanning motions of salticid retinae, enable the perception of depth by relying on a clever arrangement of the layer 1 receptors in the central fovea. Different parts of layer 1 are positioned on a ‘staircase’ at different distances from the lens, so any object, whether only a few centimetres or many metres from the eye, will create an in-focus image on some part of the layer-1 staircase. This means that the salticid can sweep and rotate the staircase of each retina across the image generated by the corneal lens and assess the distance of the object according to where on the staircase the image is in focus. However, as the depth of field at all points on the staircase was estimated to be at minimum of 3-6 cm away (Blest et al. 1981), it can be dismissed as the sole process of range-finding, as the spiders assess depth accurately at less than those distances.

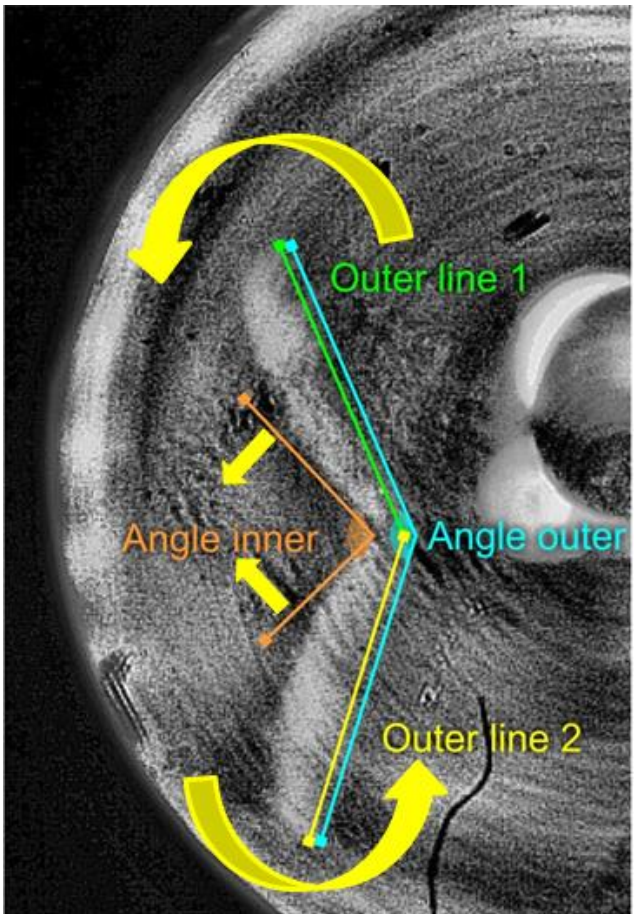

Figure 9: Illustration of the two types of retinal movements correlated with the depth of the stimulus as identified in the eye-tracker.

It is quite possible that the novel type of motion identified here provides the missing information regarding why these retinal movements correlate to depth perception. The closing of the inner angle of the retina, while the outer angle is left unchanged, suggests that what we are observing is not a change in the shape of the retina, but a change in what part of the retina we are seeing and how it is illuminated. While due to technological limitations I could not verify the exact motion that might have caused this, it is most likely that the change in illumination is the result of the retina moving closer to the light source of the eye-tracker (i.e., anterior-posterior movement), with the retina moving towards the light source (distal motion) for stimuli that are further away.

Anterior-posterior motion of the retina could theoretically be achieved by bilaterally contracting the muscles surrounding the eye-tube, causing a shortening the axial length of the eye. Indeed, the torsional motion (as seen by the movement of the outer angels of both arms of the retina) and the anterior-posterior motion could be part of the same motion (as suggested by Land 1969b) where, by twisting the eye-tube, its length is slightly contracted. The result of such motion would be the ability to accommodate to the distance of objects in the visual field by modifying the distance between the (static) corneal lens and the retina. This is the method of accommodation seen in fish and amphibians (Harkness 1977; Kawasaki et al. 1988; Wagner and Schaeffel 1991; Ott 2006), except that in fish, it is the lens that moves (without modifying its focal length) rather than the retina. Indeed, Blest et al. (1981), calculated that a mere 2 µm difference in the axial-length of the eye-tube (from 655 µm to 657 µm in *Plexippus validus*, which is now redescribed as *Servaea incana*, one of the species tested here) results in a depth of field difference from 6 cm-∞ to 3 cm-∞. By slight further adjustments of the axial lengths the depth of field could be extended to include distances much nearer to the animal, finally explaining the ability of salticids to make accurate judgments at such short distances. These differences in the depth of field are consistent with our finding that the retina appears to be further away for near stimuli.

The possibility of the visual accommodation by changes in the axial length was further corroborated by a computational model of salticid optics based on the matrix optics method (see Dagg and Vanderkooy 1973, Appendix 3) using measurements of two different salticid species from Land (1969a), Williams and Mclntyre (1980) and Blest et al. (1981). The model (Figure 10) shows an exponentially decreasing function between the axial length (X-axis) and the focusing distance (i.e. depth of field, Y-axis). It is important to note that these models are by no means accurate, as many of the variables needed for the model were not available and were assumed or derived (see Appendix 3). Nonetheless, in both calculations, the region with the most extreme derivative (i.e., strongest effect on focusing distance with the minimal change in axial length) is within the ballpark of the assumed axial length of the species (*P. johnsoni,* ~670µm; *P. fimbriata* ~1660µm).

Further strengthening this hypothesis is that the tissue of the eye tubes is very soft. Indeed, it is this fact that prevents direct measurements of the axial length, as the tissue is readily damaged when frozen (Blest et al 1981). The existence of accommodation in salticids was originally dismissed by Land (1969a), who could not find any differences in the axial length across different species. However, in his measurements using an opthalmoscope, Land (1969a,b) waited for the retinae to cease moving and reach a resting position. As such, it would have been highly unlikely for him to identify any differences in the axial length. Nevertheless, Land (1969b) did, in fact, suggest that one of the two possible functions of two of the six muscles surrounding the eye-tubes would be to change the length of eye-tube.

An interesting possible outcome of accommodation in salticids is the effect it would have of the properties of the foveal pit at the distal tip of the eye-tube. Williams and McIntyre (1980) described the telescopic properties that emerge from the pit lens by calculating the magnification of an image as a function of the lens properties and the distance between the retina and the lens:

$$m = \frac{size\ of\ i}{size\ of\ i'} = \left[1 + \frac{P_P\left(1 - \bar{d}P_L\right)}{P_L}\right]^{-1}$$

Where *i* is the size of the retinal image of an object post-magnification, *i'* is the size of an image before magnification, P_p and P_L are properties of the pit lens and corneal lens accordingly, and $\bar{d} = d/1.336$, where *d* is equal to the distance between the corneal lens and the pit lens and 1.336 is the reflective index of saline. What this means is that as *d* decreases, so does the magnification, resulting in a smaller retinal image of the object. However, another outcome could be that the contraction of the eye-tube also results

in changes to the properties of the foveal pit, should its shape alter. Furthermore, and perhaps of most significance, accommodation would also expand the function of the foveal pit, enabling it to act as a focus indicator (Williams and McIntyre 1980), as is the case in some vertebrates, such as falcons (Harkness and Bennet-Clark 1978). Such a focus indicator acts as a special system for the rapid indication of the direction of focus error, enabling faster accommodation responses (Harkness and Bennet-Clark 1978), further enhancing the incredible visual capabilities of these animals. Unfortunately, without the ability to measure the actual changes in axial length, and any commensurate changes of accommodation on the physical and optical properties of the foveal pit, it is difficult to make further predictions.

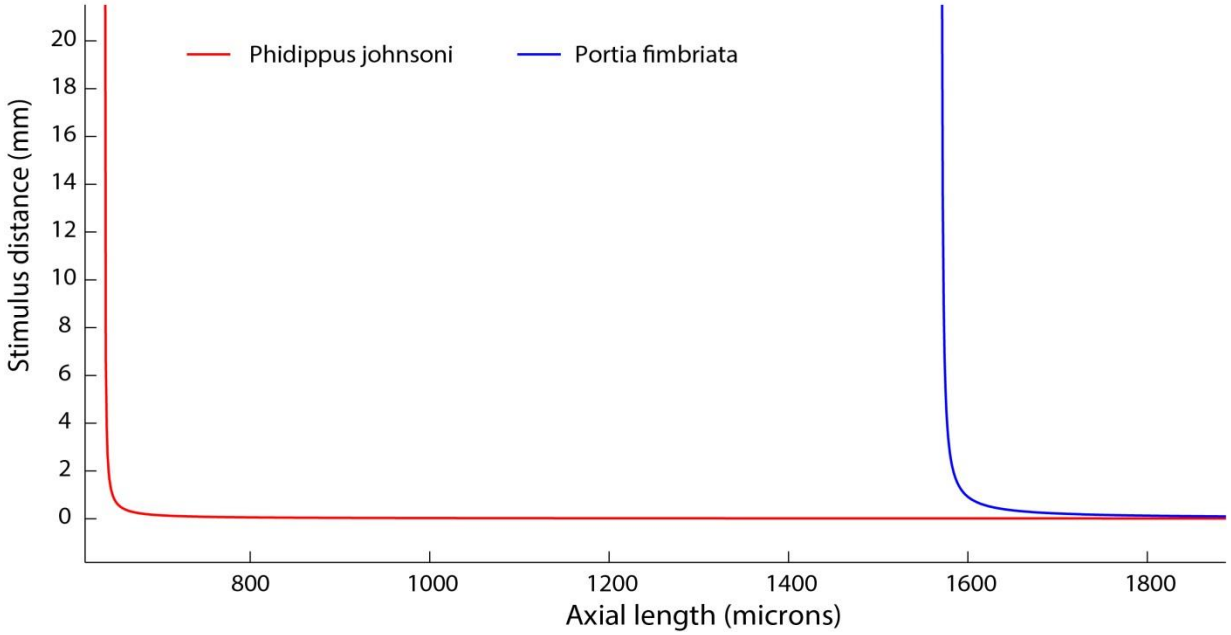

Figure 10: Model of salticid principal eye optics using measurements from two different species, showing a negative exponential relationship between the axial length of the eye-tube and the focus point.

(d) *Conclusions*

Taken together, these experiments exemplify just how much we still have to learn about this remarkable visual system. These experiments tested depth perception over a wide range of biologically-relevant distances, from about 5 to 250 mm. The flying saucer experiments were based on distances between 30 and 50 mm, while the wavelength experiments studied depth perception at distances between 5 and 10 mm and the eye-tracker experiments simulated distances between 70 (the minimum apparent distance for which the eye-tracker can be adjusted) to 250 mm.

By disproving the use of stereopsis and failing to support depth perception through image defocus and chromatic aberration at short to mid-range distances, we have shown that the processes of monocular

depth perception in salticids are more complex than they initially seem. The novel finding that retinal movements correlate with depth, which we suggest might indicate some form of visual accommodation, further complicates matters. Nevertheless, the suggestion of retinal accommodation aligns with what we know regarding the visual acuity, the telescopic structure of the eye, and the behavioural characteristics of salticids. Ultimately, it is likely that, as in many other complex systems, no one method of depth perception works in isolation.

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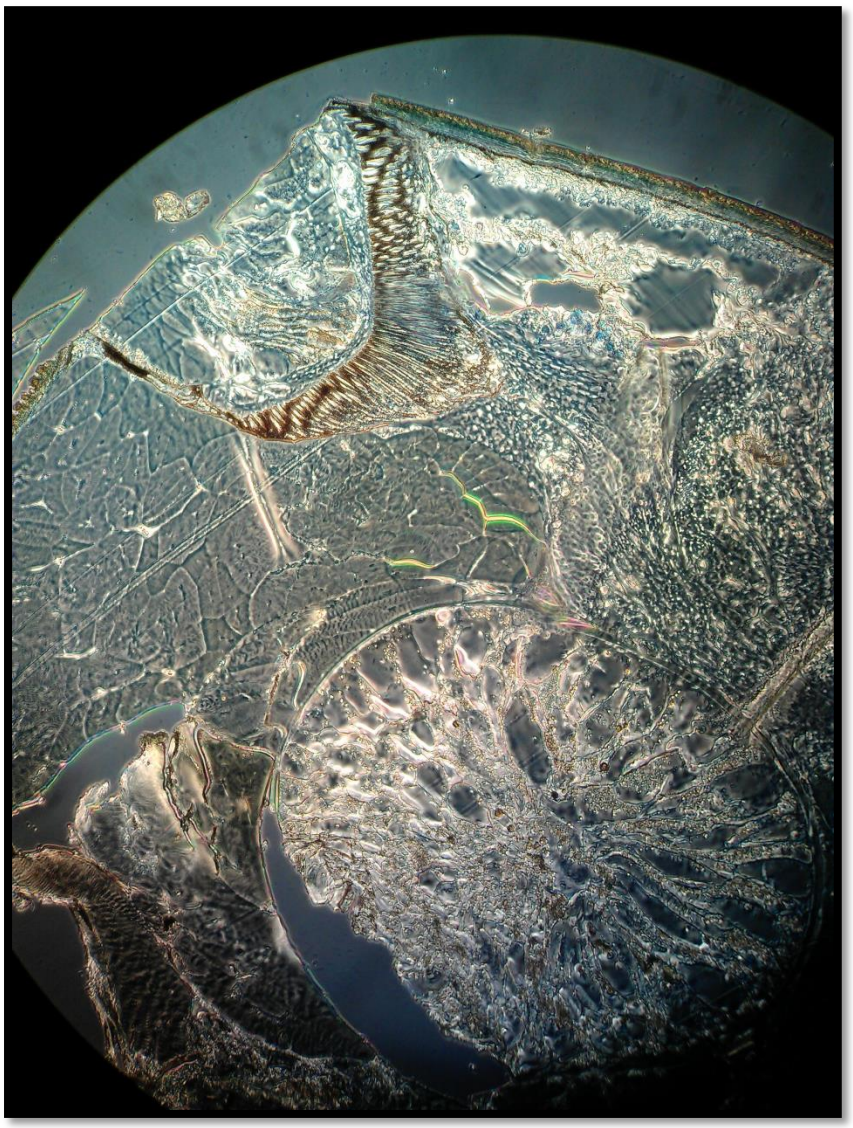
Chapter 7

Discussion



Light microscope image from a sagittal section through the AL eye of the cephalothorax of Marpissa marina*. The concave retina of the AL eye can be seen on top left of the image* (Preparation, fixation, staining and photo: Y. Dolev)

“It's not what you look at that matters, it's what you see.”

Henry David Thoreau

Synopsis of main findings

In this thesis I investigated different aspects of visual perception in jumping spiders (Salticidae). This work has been performed at various levels, starting with the end result of perception – behaviour, working down through to anatomical processes and suggesting possible neurobiological pathways that may underlie some of these behaviours.

My thesis is divided into three main sections following from the general introduction (Chapter 1). In the first section (Chapters 2 and 3), I used salticid behaviour and prey-choice decisions to investigate object recognition and visual categorisation. I also investigated the role that predatory specialisation plays in these processes. Surprisingly, I found that the highly specialised predator *Evarcha culicivora* seems to 'bypass' a conceptual step of visual processing in order to gain rapid object recognition. This step is the assessment of the conglomeration of the parts of a visual image into a whole. As a result, the specialist predator did not differentiate between a stick figure of its preferred prey and a scrambled version of the same stimulus, while a generalist predator (in this case *Hypoblemum albovittatum*) easily distinguished between the two. Although this adaptation has the potential to create false-positive recognition under laboratory conditions, it is less likely to do so in nature, as the combination of both the set of shapes at the correct relative angles, and a lack of any additional information (visual or from any other sensory modality) which could affect the perceptual process makes false-positive recognition unlikely to occur. Perhaps most surprisingly, this work revealed the ability of *E. culicivora* to identify and compare abstract features in visual images – the hypothetical angles formed between non-touching elements.

In the second section (Chapters 4 and 5) I introduced the use of the new salticid eye-tracker as a tool for investigating primary, Anterior Median (AM), eye retinal movement in salticids. In these chapters I showed how, despite a 'edge detection' mechanism being used by all species, retinal scanning motions are, nevertheless, strongly dependent of the stimulus being presented, as well as on the species of the spider (Chapter 5). Following this, I showed that the retinal scanning patterns are strongly affected by priming of the Posterior Lateral (PL) eyes, suggesting that working memory is accessed when the AM eyes are searching for specific stimuli. This is apparent as, following priming, the AM eye search patterns appeared to `matched the shape of the primed stimulus even when that stimulus was not being displayed to either the PL or the AM eyes. The results of the second section imply that one of the main roles of the AM eyes is the acquisition of detailed visual information guided by the secondary eyes, with the most important of these likely being the Anterior Lateral (AL) eyes due to their frontally-facing visual fields which overlap those of the AM eyes, as suggested by Zurek and colleagues (Zurek et al. 2010, Zurek and Nelson 2012).

The third and final section (Chapter 6) is built as a set of experiments using very different methodologies (from behaviour through to eye-tracking) to address one of the most contested aspects of salticid vision – depth perception (e.g., Forster 1979). In this work I disproved stereopsis as a necessary modality for depth perception, whether mediated either by the AL or AM eye retinal movement (which may create a region in which the fields of view overlap). I also failed to find behavioural support for depth perception through chromatic aberration or image defocus, as suggested by Nagata et al. (2012, 2013). Of greatest impact in this chapter is the novel finding that retinal movements appear to be correlated with depth, suggesting an additional dimension of retinal movement previously assumed not to exist (Land 1969a). This movement is the shortening and lengthening of the axial length of the AM eyes, indicating some form of visual accommodation.

While the work in this thesis focuses on visual perception, there is an underlying theme regarding the effects of the evolution of predatory specialisation on visual perception. Specifically, I utilise predatory specialisation as a tool to investigate visual perception under different conditions. Predatory specialisation is the evolution of specific behavioural traits by a predator for hunting a specific species of prey which is usually, although not necessarily, that predator's preferred prey (which is the prey the spider will choose in preference to other when given a choice; Fox and Morrow 1981, Huseynov et al. 2005, Huseynov et al. 2008). Within the Salticidae, there are many examples of such specialisations, even though most salticid species studied to date are polyphagic, opportunistic predators (Jackson and Pollard 1996, Nelson and Jackson 2011a).

In the case of the species I have used in this thesis, *Portia africana* specialises at hunting other spiders (araneophagy) using several tactics, including cryptic stalking, trial-and error aggressive mimicry of web-spider prey, and smokescreen behaviour when wind vibrates the webs of spider prey (Jackson and Hallas 1986a, Jackson and Hallas 1986b, Wilcox et al. 1996, Jackson et al. 2008b, Jackson and Nelson 2011, Nelson and Jackson 2011b, Nelson and Jackson 2011a). *Evarcha culicivora*, in contrast, specialises at hunting female *Anopheles* mosquitoes, and in the case of juveniles, they hunt this specific prey by detouring in order to approach the *Anopheles* from behind and jump up to bite it from under the thorax of the mosquito (Jackson et al. 2005a, Nelson et al. 2005). This behaviour is coupled with a strong preference for female blood fed *Anopheles* mosquitoes (Jackson et al. 2005b, Nelson and Jackson 2006, Nelson and Jackson 2012a, Nelson and Jackson 2012b). Indeed, their diet has evolved in such a way that the lack of a blood meal severally decreases their chances of survival as young (Deng Chan pers. comm). The third specialist predator used in this thesis is *Sandalodes bipenicillatus*. Although very little work has been done on this species, it specialises at hunting ants (Nelson and Card 2015), a group rarely preyed upon by spiders

as they are extremely dangerous prey for spiders (Nelson et al. 2004). Like other ant-eating salticid specialists (e.g., Jackson et al. 1998), their hunting behaviour consists of jumping on the ant and accurately pinning it down and biting the ant directly behind the head (Ximena Nelson, pers. comm.). In the behavioural work that I conducted here, these specialised behaviours and prey preferences were exploited as a mechanism for the spiders to communicate to me, through prey-specific behaviour, perceptual aspects of what it is that is recognised as preferred prey. In the eye-tracker work, specialised behaviour and prey preferences formed a basis for comparing the evolutionary effects of specialisation on the processes leading to object categorisation and recognition.

That these animals have evolved specialisations that affect not only their behaviour - but their visual processes as well - brings in to question the prevailing, if somewhat unconscious, notion that jumping spiders are a relatively unvarying group of 'automatons' subject to simple instinct-driven behaviours, even though signs to the contrary were already being noticed over a century ago, as depicted somewhat romantically by Darwin's friend George Romanes (Romanes 1886) when he stated "*The emotional life of spiders, so far as we can observe in their actions, seems to be divided between sexual passion (including maternal affection) and the sterner feelings incidental to their fiercely predatory habits*". Spiders as a whole are now dramatically altering the point of view of this group being simple and perhaps inflexible (e.g., Morse 2000,Seah and Li 2001, Uetz et al. 2016). Much of this change in attitude, at least among scientists, is due to the many salticid examples showing that spiders display significant cognitive abilities (such as learning and problem-solving), despite their small nervous systems (Jackson and Wilcox 1993c, Jackson and Wilcox 1993a, Wilcox and Jackson 1998, Tarsitano and Andrew 1999, Nakamura and Yamashita 2000, Jackson and Carter 2001, Jackson et al. 2001a, Harland and Jackson 2004, Jackson and Li 2004, Jakob et al. 2007, Jakob and Long 2016).

Nevertheless, by and large, salticids are still viewed as a closely-related group with minimal variation, despite ample evidence of the profound species-specific differences in their behaviour and life histories (Jackson 1978, Cutler 1982, Jackson 1985, Logunov 1997, Punzo 2000, Bartos 2005, Ceccarelli and Crozier 2007, Jackson et al. 2008a, Jackson et al. 2008b, Pekar and Jarab 2011). Most of these differences, however, are largely considered to be 'exceptions to the rule'. This is somewhat surprising considering that the Salticidae have over 5800 (and counting) described species in over 580 genera and are overwhelmingly the largest and most diverse spider group (Maddison 2015, Platnick 2015). Indeed, salticids are at least as diverse (and as ancient) as birds (Hill and Richman 2009) and, despite being poikilothermic, are found in a surprisingly wide breadth of habitat types in all non-polar terrestrial ecosystems (Maddison 2015), including hot dry deserts (Jackson and Li 1998, Li et al. 1999, Richman et al. 2011, Bustamante et al. 2014),

swampland and jungles (Muma 1973, Wanless and Lubin 1986, Cutler and Edwards 2002, Zhang et al. 2003), and alpine environments and tundra (Wanless 1975, Cutler 1982, Logunov, 1997).

Alongside the variation in habitat types in which they are found, salticids exhibit an incredible variety of lifestyles. For example, *Portia fimbriata*, a closely related species to the *P. africana* used in this study, is a 'primitive' salticid from Australia that exhibits geographical variation in its araneophagy, as the *P. fimbriata* from Queensland specialise on eating salticids in addition to other types of spiders (Jackson and Wilcox 1990, Jackson and Wilcox 1993b, Jackson and Wilcox 1993a). As may be expected, it is extraordinarily well adapted for hunting these dangerous, and in the case of other salticids - highly visual - prey (Harland and Jackson 2004). As an araneophagic "poly-specialist" (Nelson and Jackson 2011a), *P. fimbriata* invades the webs of different spider species, with a specific strategy for each type of prey. *P. fimbriata* is tasked with identifying which particular spider inhabits a given web, and which approach strategy should be adopted. These capabilities have likely coevolved with the extreme spatial acuity of *Portia*'s eyes (Jackson and Blest 1982).

Other examples of unexpected life histories that are found among salticids include *Euophrys omnisuperstes* (which translates to "standing above everything"), which is the highest known permanent resident on earth, living above 6700 m on the slopes of Mount Everest, where it feeds on insects that wind carried up from lower altitudes (Wanless 1975). A final example of unusual feeding behaviour is *Bagheera kiplingi*, aptly named after the pacifist panther from Kipling's "*Jungle Book"*, which is the only spider with a largely herbivorous diet (although numerous salticids are now known to feed on plant material, particularly nectar, in addition to live prey, Jackson et al. 2001b, Taylor and Pfannenstiel 2008). However, *B. kiplingi* is not a 'dull' grazer; in typical salticid fashion, it uses speed, stealth and evasion to snatch nutritious plant nodules away from ants (Meehan et al. 2009).

Considering the diversity found among salticids, despite the relatively limited literature on these spiders, it is not surprising that different species evolved profound differences in their visual capabilities, and even the specific eye structures and neuroanatomy (Eakin and Brandenburger 1971, Blest and Sigmund 1984, Blest and Carter 1987, Blest et al. 1988, Blest et al. 1990). Recent work has now begun to show how particular adaptations fit in with the specific visual needs of a salticid within its particular ecological niche (Zurek et al. 2015).

It is because I wanted to capture the inherent variation in this family of spiders that in this thesis I used a number of distantly related species. Unsurprisingly, many of the differences in visual behaviour that I found depended on the species of the spider being tested. For example, the evolution of specialisation appears to have affected the processes of object categorisation (Chapter 2), in this case specifically leading

the predatory specialist (*Evarcha culicivora*) seeming to have a 'shortcut' template for rapid visual assessment of biologically-salient prey stimuli (Chapter 3).

The mechanism whereby this type of assessment might happen could be based initially on the retinal movements of the AM eyes. Although I was unable to test *E. culicivora* in the eye-tracker, as this species is from Africa and the population in the lab in New Zealand had crashed by the time that I began the eye-tracker work, it is evident that, while there are commonalities, the basic retinal movements are strongly dependent on the species (Chapter 4). Based on the work I did in Chapter 5, it seems likely that the process of extraction of key visual information typically begins prior to the stimulus being analysed by the AM eyes. Indeed, with the secondary eye visual fields covering almost 360 degrees, and that of the AM eyes being rather restricted to the frontal fields of view, it is likely that the majority of stimuli will be perceived by the secondary eyes.

The priming experiments using the eye-tracker revealed that the secondary eyes do appear to provide information regarding shape that facilitate the scanning pattern enacted by the AM eyes once the stimulus in their visual field. Furthermore, it seems that this information, sent from the secondary eye retina to the primary eye muscles, requires at least one pathway that involves high-order processing (Chapter 5). In chapter 6 I replicated previous work (Nagata et al. 2012), demonstrating the power of this underused aspect of science in behavioural work. The simple fact that I used a different species may explain why I got very different results to those of Nagata et al. (2012), but, based on further experiments which led to my finding that salticids may use accommodation to help in their depth judgments, I am confident that chromatic aberration is not an important mechanism whereby these spiders perceive depth using monocular cues. Indeed, some of the fundamental assumptions behind this reasoning, including their inability to perceive red light, are being demolished with further studies (Zurek et al. 2015). As more work is being done on spider vision we are discovering that some of our most basic tenets in the area are wrong (Land and Barth 1992, Schmid 1998, Fenk et al. 2010, Fenk and Schmid 2010,2011), and I expect this pattern to continue.

Future directions

More than anything else, this work has exemplified just how much more we could do in this area with the tools we now have. I list but a few examples.

In chapter 2 I show that the inter-element angles are an important, though hidden, aspect of the stimulus that leads *E. culicivora* to the recognition of an *Anopheles* mosquito. This was revealed because,

when using two stimuli that only differ in the inter-element angles, the spiders distinguished between them and preferred the stimulus with the angles that matched the body angles characteristic of the resting posture of *Anopheles*. However, the extent by which the angles and the configuration of the scrambled '*Anopheles*' stimuli can be modified while still maintaining the spiders' preference for it (i.e., the plasticity of the recognition processes in *E. culicivora*) has been left unanswered. This is primarily due to the fact that I was no longer able to obtain any more *E. culicivora* from Kenya. Should work continue on this species it would be most rewarding to test the extent of the plasticity of categorisation of stimuli in *E. culicivora* in response to different inter-element angles. Further, investigating the retinal movement of this species through the use of the eye-tracker not only on the set of stimuli presented in Chapter 4, but also on the different *Anopheles* stick figures from Chapters 2 and 3 would be of considerable interest. These data could shed light on whether it is the AL eyes or the AM eyes that look for the body part angles; if this is done primarily by the AM eyes, then the eye-tracker would show the retinas following the edges of each of the different elements of the stimuli. Failure to do so would mean that the angle data cannot be collected by the AM eyes, meaning that it is the only other eyes with a frontal field of view, the AL eyes, which must be responsible for this aspect of the image analysis.

While the data presented in Chapter 4 are interesting and revealing, for me they create many more questions than they actually answered. In effect, this study serves mainly as an introduction to a powerful new tool and methodology. Of the many questions to arise while using the eye-tracker, was the one that I addressed in Chapter 5, regarding priming of the AM eyes by the secondary eyes. Even though I touched on this subject, years of potential research with the eye-tracker lie ahead of us.

One of the stand-out features of the salticid visual scanning is the surprising preponderance of use of the retinal arm edges during visual scanning of stimuli. This leads to questions such as:

- In what proportions are each part of the retina used?
  Given a set of scanning motions (from stimulus presentation untill the AM eyes cease movement), what proportion of time did the spiders use their fovea to scan the stimulus? What proportion of time did the spiders use the top or bottom retinal arm to scan the stimulus?
    - How do these proportions change between different species/different stimuli?
    - How do these proportions change when using more and more complex stimuli (i.e., the amount of detail in the stimuli)
- Are there any temporal correlations to the use of specific regions of the retina?
  What I mean by this is whether the spiders start scanning with the fovea and then move to the retinal arms or *vice versa*. Such data would give us insight into the steps of the cognitive processes

of constructing a holistic representation of a stimulus (for example: *start with the edges, look for basic structure using retinal edges; according to this structure, look for details using the fovea*).

- Are there any temporal correlations to the scanning of different areas of a given stimulus?
  In other words, for a given stimulus, do the spiders start by looking toward the right or left of the stimulus? Do they start with corners or edges? Is there structure in the order with which they look at different parts of the stimulus?

The large variation in scanning patterns between the different stimuli and the different species leads me to suggest potential changes to future experimental design. One such change would be to introduce a common starting position of the spiders' retinae for all trials. This could be achieved by having a small vibrating dot at a specific screen location prior to each session. The slight movement of the dot should cause the spiders to rapidly focus on that point prior to the presentation of the stimulus. This would also allow us to factor the spider's level of attention in the analysis and would make answering the following questions much simpler:

- How much variation in the scanning patterns are found within species?
  In Chapter 4 I compared scanning patterns for variations between species and between stimuli, but I have not done the same analysis for a given stimulus between individuals of the same species as the N's were too low (up to six spiders for each species), in part because attention toward the stimulus could not be ascertained for some individuals. Were this the case, sample sizes could be improved.
- How much individual variation exists in the scanning patterns?
  This could be done by repeatedly testing the same spider using one stimulus. By investigating the effect of different break between testing trials (e.g., a session hour, every day, three days or a week) using different spiders we could compare the scanning patterns over time for each spider, as well as between the different break protocols. This would test for the effects of short term vs. long term memory on visual scanning patterns.
- How do hunger, sex and age affect scanning patterns?
  Here we could compare the scanning patterns between spiders of the same species, but using different sexes, ages or feeding regimes when using the same stimulus,.

These questions require an additional form of data analysis to the approach taken in this thesis. Specifically, the retinal tracks can be viewed as a spatio-temporal clustering problem. The introduction of Markov models – a model for the estimation of the distribution of the next location by the previous *k*

locations (Mathew and Martins 2012, Dietterich 2002, Nguyen et al. 2005, Vasquez et al. 2009, Mathew et al. 2012) – could prove extremely useful. Unfortunately, time constraints prevented me from pursuing this solution.

In Chapter 4 I purposefully used very simple stimuli to facilitate simpler comparisons between the different species and stimuli. This resulted in distinct and visible differences in the scanning patterns elicited by the different stimuli. However, the simplicity of these stimuli also resulted in the fact that none of the stimuli contain any detail that could not be obtained by the spatial resolution of AL eyes alone. While the potential for many more discoveries through the use of simple stimuli is unquestioned (see pervious questions in this chapter), other questions that can be answered only with more complex stimuli that only the AM eyes could perceive include:

- Given the non-unified distribution of colour sensitivities in the different layers of the retina (Devoe 1975, Blest et al. 1981, Koyanagi et al. 2008, Nagata et al. 2012, Zurek et al. 2015), how do retinal movements change when colour is introduced, specifically in terms of the two previous questions?
    - How do the sex and age of the spider play into any differences found in scanning colour images? Often male salticids are very colourful and females are drab, suggesting that colour vision may be sexually dimorphic (Zurek et al. 2015)
- What role do the AL eyes play in directing the location of the AM eye retinae?
  This could be addressed through the use of stimuli with areas of detail that the known optics of the AL eye (Blest and Sigmund 1985, Blest 1987) would predict to be seen as blurred, but which could be discerned by the AM eyes given their superior spatial resolution (Blest and Price 1984, Blest and Sigmund 1985, Blest 1987, Blest et al. 1988, Blest et al. 1990).
- In nature, background noise always exists. How does the introduction of 'visual noise' (in the form constant motion of the background, or partially hidden stimuli) affect retinal scanning motions to a known stimulus (i.e., one whose pattern is known in the absence of background noise)?

In Chapters 4 and 5 I focused primarily on the scanning motions of the AM retinae, but in Chapter 5 I found an interesting component of the saccadic motions. In all cases, the saccades appeared to have a back and forth movement. This was probably because the retinal saccadic motions occurred either during or directly before the 'body saccades' or rotation of the spiders following priming of the posterior eyes. Using the combination of the eye-tracker with simultaneous recordings of the test spiders' movement, as measured by the rotation of the polystyrene ball (e.g., Moore et al.2014) held by the tethered spiders (see Chapter 4, Appendix and Chapter 5), one could address questions such as:

- What are the characteristics of the retinal saccades (speed, size, duration), and how do they relate to body saccades?
- Do salticids display scanning motions during their own movement (i.e., during walking or running)?
- Do salticids display scanning motions during stimulus movement? This requires a clearer distinction between the tracking and scanning motions described by Land 1969a.
- What are the characteristics and limitations of tracking, as opposed to scanning, motions? Specifically, how fast can the spiders track stimuli? Do they use both retinae together when tracking? What part of the retina covers the stimulus during tracking?

A common method used when investigating visual searching is that of target 'pop-out' experiments. 'Pop-out' in visual search reflects the capacity of observers to rapidly detect certain visual targets independent of the number of distracting objects in the background (Maljkovic and Nakayama 1994, Wang et al. 1994). These visual targets typically display features sufficiently different from the background distractors which facilitate this behaviour (i.e., different colours, orientation, direction of movement, etc.). 'Pop-out' capabilities (through behaviour or single-cell recordings of visual neurons) have been shown among wide a variety of animals, both in vertebrates (e.g., Lettvin et al. 1959, Kastner et al. 1997, Woo et al. 2009, Ben-Tov et al. 2015, Dutta et al. 2016), and invertebrates (e.g., Barnett et al. 2007, Lee & Nordström 2012, Lee et al 2015).

- How would salticids handle 'pop-out' target experiments, such as:
    - Colour contrast of stimulus in noise (Baldassi and Burr 2004)

      For example, using a stimulus of a field of grey dots and one black one, do the AM retinae rapidly focus on a black dot? How about green and black?
    - Orientation contrast of stimulus in noise (Nothdurft 1991)

      For example, using a stimulus of a field of vertical bars with one horizontal bar, do the AM retinae rapidly focus on horizontal bar?
    - Scene-relative stimulus movement (Rushton et al. 2007, Woo et al. 2009, Ben-Tov et al. 2015)

      For example, using a field of dots moving from right to left with one dot moving from left to right, do the AM retinae track the odd one out?
    - Priming of pop-out features (such as the colour or orientation of the stimulus) through either repetition of the stimulus or, like in Chapter 5, by flashing the pop-out features to the PL eyes prior to displaying the stimulus to the AM eyes (Maljkovic and Nakayama 1994, Wang et al. 1994)

The work presented in Chapter 6 leaves unanswered the cause of the retinal movements that are correlated with depth perception. In other words, are the rotation and closing of the retina which were observed the result of anterior-posterior motion? While my suggestion of visual accommodation through changes in the axial length seems likely, this remains to be proven and can not be done using an eye-tracker. My thought is that, given time, this could be achieved through the methods outlined below:

- Externally recording the retinal movements using two high-speed IR cameras with high magnification and resolution. The retinae of the spiders can be illuminated by using two high-intensity narrow-beam cold light sources pointed into the spiders' cephalothorax. The use of this type of illumination for externally recording retinal motions has proven feasible (Elizabeth Jakob and Skye Long pers. comm.). By simultaneously recording the retinal movements both laterally and dorsally, extrapolation of retinal movement in all three dimensions is theoretically possible given high enough magnification and resolution.
- *In vitro* stimulation of the eye-tube muscles could replicate retinal movements. By combining the stimulation of all or part of the muscles surrounding the eye-tube (see Land 1969a), any potential changes in the axial length could be revealed, replicated and measured.
- Couple the *in vitro* stimulation of the eye-tube muscles with electrophysiological recordings from the first neuropil, as well as from AM eye layer 1 photoreceptors (Hill 1975, Menda et al. 2014), while presenting a stimulus at different distances from the lens. What we would be looking for are differential responses that correlate between the length of the eye-tube and the distance of the stimulus.

Finally, during my PhD, two papers were published with findings or methodologies that I believe could be combined with the eye-tracker, potentially yielding fascinating results. The first of these I discussed in Chapter 4:

- Peckmezian and Taylor (2015) published a study showing the first virtual reality (VR) system for salticids, showing how this tool could be used for analysing navigation. I suggest combining VR with the eye-tracker, to not only assess navigation, but also how the spiders view their visual environment, which ultimtaley leads to the descision-making involved in navigation.
- The same VR system can be used to control stimulus movement in the eye-tracker in accordance to the spider's movement. This would overcome any potential visual conflicts that arise from the

spider moving but without the stimulus responding accordingly (by 'tracking' the body motion of the spider).

The second paper that was published that I believe could add depth to the use of the eye-tracker is that of Zurek et al. (2015), who published a study showing that some salticids have a spectral filter in part of the layer 1 AM eye retina which enables trichromatic vision.

- Using two different salticid species, one that has this spectral filter and another that does not, we could compare their retinal scanning movements by showing them two versions of the same stimulus, one in grey-scale and another including red. I would hypothesise that the species with no spectral filter should show no differences in the scanning patterns between the two stimuli, while the species with the spectral version would show differences. These differences could shed light on the use of retinal motions that might be specific for obtaining colour information from a stimulus for which this might be important (e.g., recognition of a rival or a mate, see Zurek et al. 2015).

The recent breakthrough of being able to record multiple (Menda et al. 2014), and even single, unit neural responses to stimuli from visual areas in the brain and optical processing system of salticids, coupled with novel tools for the study of spider vision will see a dramatic change in perspective regarding the processes of salticid vision in the next ten years. I suspect that this perspective will cement the importance of evolution of visual perception, not only in spiders, but will provide important insights into the flexibility and adaptiveness of visual systems as a whole. Concomitant with this improvement in our knowledge will be our understanding of the cognitive processes that arise through perception.

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Appendix One

Chapter 2: Innate pattern recognition and categorisation in a jumping spider - supplementary information

Video S1: Sample stimulus video for the two-choice predatory behaviour experiment presenting images 3 and 4. **Link Here**

Video S2: Sample session video from the two-choice predatory behaviour experiment. **Link Here**

Table S1: Results from the single-choice predatory behaviour experiment (all spiders).

Stimulus	N	% Noticed	Notice distance (cm) M/IQR	% Stalked	Stalking initiation distance (cm) M/IQR	Decision time (s) M/IQR	% Pounced
	33	82	6/5-7	74	5.5/5-6.8	5/2-24	90
	32	88	6/3.6-7	64	5.75/4-7	13/3-39	67
	30	90	6.5/5.5-7	74	5.8/4.1-6.9	10/4-21	75
	37	81	6.5/5.5-7.1	77	6/5.5-6.5	18/4-49	87
	32	84	5.5/4-7	56	5/3.5-6	14/2-32	73
	32	84	5.5/5-6.5	30	5/3.1-6.5	10/2-18	88
	40	73	8/5.3-9.8	17	5/3.5-7	8/4-10	80

M = Median, IQR = interquartile range. The percentages of the spiders that stalked/pounced are nested within the percent of spiders that noticed/stalked, respectively.

Table S2: Statistics comparing between the different stimuli for the single-choice predatory behaviour experiment (results from all spiders; data in Table S1).

	Notice	Notice distance	Stalk	Stalking initiation distance	Decision time	Pounce
Statistic	*6.71	**1.303	*37.87	**6.654	**3.928	*4.00
p	= 0.349	= 0.972	< 0.001	= 0.354	= 0.686	= 0.677

*Cochran's Q; **Friedman's test (χ^2); df = 6 in all tests.

Table S3: Results from the single-choice predatory behaviour experiment (female spiders).

Stimulus	N	% Noticed	Notice distance (cm) M/IQR	% Stalked	Stalking initiation distance (cm) M/IQR	Decision time (s) M/IQR	% Pounced
	11	82	6/4.8-7	67	5.5/4.8-6.6	13/2-25	100
	10	90	6/3.8-8	67	5.5/3.9-7.6	9/3-61	67
	9	100	7/6.8-8	67	6.75/5.1-7.8	15/8-156	83
	25	80	6.5/5.5-7.5	90	6/3.8-8.5	17/5-143	89
	9	100	6/3.5-7.3	56	4/2.8-8.5	14/11-29	80
	10	90	6.5/5.3-7.5	44	6/3.88-8.5	17/5-142	100
	23	87	8.8/6-10.8	15	5/2-5	9/8-9	67

M = Median, IQR = interquartile range. The percentages of the spiders that stalked/pounced are nested within the percent of spiders that noticed/stalked respectively.

Table S4: Statistics comparing between the different stimuli for the single-choice predatory behaviour experiment (results from female spiders; data in Table S3).

	Notice	**Notice distance**	**Stalk**	**Stalking initiation distance**	**Decision time**	**Pounce**
Statistic	*6.857	**4.409	*14.195	**4.233	**2.747	*5
p	= 0.334	= 0.621	< 0.05	= 0.645	= 0.84	= 0.544

*Cochran's Q; **Friedman's test (χ^2); df = 6 in all tests.

Table S5: Results from the single-choice predatory behaviour experiment (male spiders).

Stimulus	N	% Noticed	Notice distance (cm) M/IQR	% Stalked	Stalking initiation distance (cm) M/IQR	Decision time (s) M/IQR	% Pounced
	8	75	5/2.4-7.5	50	5/*	6/*	67
	8	88	3.5/3-6	43	5/*	43/*	33
	9	78	6/4.5-6.5	57	4.5/3.8-5.6	8/1-39	25
	9	89	6.5/5.6-7	50	5.75/5.5-6.8	31/8-39	75
	8	88	5/3.5-6.5	29	5.5/*	21/*	50
	9	67	5.5/3.6-7.6	17	2.5/*	2/*	0
	17	53	6/2-8.8	22	5.5/*	4/*	50

M = Median, IQR = interquartile range. The percentages of the spiders that stalked/pounced are nested within the percent of spiders that noticed/stalked, respectively. *Insufficient cases for IQR.

Table S6: Statistics comparing between the different stimuli for the single-choice predatory behaviour experiment (results from male spiders; data in Table S5).

	Notice	**Notice distance**	**Stalk**	**Stalking initiation distance**	**Decision time**	**Pounce**
Statistic	*3.778	**11.095	*5.636	***	***	***
p	= 0.707	= 0.085	= 0.465	***	***	***

*Cochran's Q test; **Friedman's test, $\chi 2$; ***Insufficient cases for analysis; in all tests, df = 6.

Table S7: Results from the single-choice predatory behaviour experiment (juvenile spiders).

Stimulus	N	% Noticed	Notice distance (cm) M/IQR	% Stalked	Stalking initiation distance (cm) M/IQR	Decision time (s) M/IQR	% Pounced
	14	86	6.3/5.1-7	92	5.5/5-7	4/3-11	91
	14	86	6.5/4.5-7.4	75	7/4.5-7	8/4-26	78
	12	92	6/4.5-7	91	5.5/4-6.6	7/5-15	90
	3*	67	3.75/*	50	4/*	334/*	100
	15	73	5/4-6.5	73	5/3.6-5.8	10/2-30	75
	13	92	5.3/5-6	25	5/*	4/*	100
	**	-	-	-	-	-	-

M = Median, IQR = interquartile range. The percentages of the spiders that Stalked/Pounced are nested within the percent of spiders that Noticed/Stalked respectively. *Insufficient cases for IQR. **No juveniles tested with this stimulus.

Table S8: Statistics comparing between the different stimuli for the single-choice predatory behaviour experiment (results from juvenile spiders; data in Table S7).

	Notice	**Notice distance**	**Stalk**	**Stalking initiation distance**	**Decision time**	**Pounce**
Statistic	*2.545	**6.101	*14.261	**4	**7.467	*4
p	= 0.637	= 0.192	< 0.01	= 0.406	= 0.113	= 0.406

*Cochran's Q test; **Friedman's test (χ^2); in all tests, df = 5.

Table S9: Results from the single-choice predatory behaviour experiment for each sex/age group.

Sex/ age	N	% Noticed	Notice distance (cm) M/IQR	% Stalked	Stalking initiation distance (cm) M/IQR	Decision time (s) M/IQR	% Pounced
F	97	88	7/5.5-8	56	6/4.5-7	15/4-32	85
M	68	74	6/3.9-7	38	5/4.5-6	13/2-40	53
Juv	71	85	5.8/4.5-7	70	5/4-7	7/3-19	86

M = Median, IQR = interquartile range, F = female, M = male, Juv = juvenile. The percentages of the spiders that stalked/pounced are nested within the percent of spiders that noticed/stalked, respectively.

Table S10: Statistics comparing between different sex/age groups for all stimuli in the single-choice predatory behaviour experiment (data in Table S9).

	Notice	Notice distance	Stalk	Stalking initiation distance	Decision time	Pounce
χ^2	5.762	14.021	11.29	3.341	3.699	10.461
p	= 0.056	< 0.005	< 0.005	= 0.188	= 0.157	< 0.01

Kruskal-Wallis tests (df = 2). In pairwise analysis females noticed the stimuli from significantly further away than other groups (Table S11)

Table S11: Differences between the sex/age groups in notice distance, stalking propensity and pouncing propensity for all stimuli in the single-choice predatory behaviour experiment (data in Table S9)

	Notice distance			**Stalk**			**Pounce**		
	F vs M	**F vs J**	**M vs J**	**F vs M**	**F vs J**	**M vs J**	**F vs M**	**F vs J**	**M vs J**
U	1471	1751	1486	1732.5	2205	1020	-306.5	1005	-267
p	< 0.005	< 0.005	= 0.738	< 0.05	= 0.099	< 0.001	< 0.01	= 0.968	<0.01

Pairwise analysis of differences in noticing distance, stalking propensities and pouncing propensities once stalking was initiated. Mann-Whitney U tests. F = female, M = male, J = juvenile.

Appendix 2

Eye-tracker supplementary information

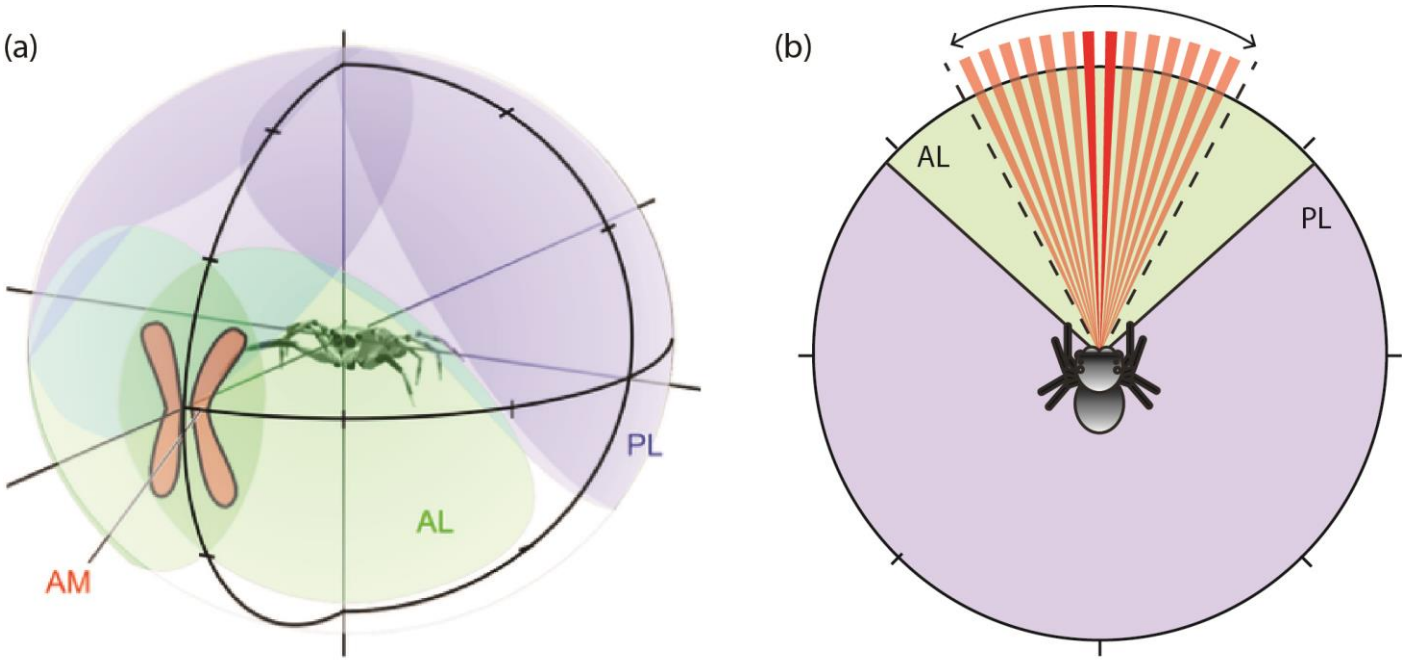

Figure S1: Typical fields of view of the three main eye pairs of a salticid spider (Anterior Median or AM (red), Anterior Lateral or AL (green), Posterior Lateral or PL (purple)). (a) Boomerang-shaped AM or principal eye retinae fields of view lie within the overlapping fields of view of the AL eyes. (b) Dashed lines indicate range of movement of the AM eye tubes. (b) Redrawn with permission from Daniel Zurek.

Eye-tracker

The spider eye-tracker is a specially designed instrument to enable the recording of retinal activity of jumping spiders in near-infrared light through an ophthalmoscope while simultaneously displaying digital stimuli (in visible light). Stimuli were projected using an AAXA M2 Micro Projector, and retinal movements were recorded with a PixeLINK IR camera (PL-B771F Aptina) at 30 frames per second. The eye-tracker used in this study (Figs. S2, S3) is a slightly modified version of the eye-tracker described in Canavesi et. al. (2011). Three major modifications were introduced to the eye-tracker used in this study:

- An iris (taken from the condensing stage of an old light transmission microscope) was added in front of the IR camera to filter out excess light (Figure S4).
- A 3D printed 'theatre' was created and attached to the input port to ensure stimuli had consistent magnification and properties. The theatre had three slots in which the screen could be placed, allowing for three pre-defined depths of stimulus presentation. For this study, the theater and projector were placed on a separate moveable platform (Figure S2).

- The light source was moved 200 mm backwards by adding a copper extension tube and introduced a 660 nm filter to disperse the beam.
- The optical mask holder (Figure S5), which is used to reduce excess light beaming onto the spiders (while not impairing the stimulus), but which caused reflections in the system, was slightly enlarged to accommodate for the mask used in this study. The masks were opaque glass slides with precise clear windows in the opaque layer created using nanolithography. Different dimensions and spacing of the windows allowed for adaptation for different spiders. All mask slides used here were designed in the spider lab and were made by the Department of Electrical Engineering at the University of Canterbury.

Tethered spiders were placed in a hexadimensional manipulator (which allows control of the pitch, yaw and roll of the spiders (Figures S6, S7), which, in turn, was connected to a micromanipulator, enabling control of the XYZ axis for fine placement of the spider in front of the eye-tracker (Figure S3).

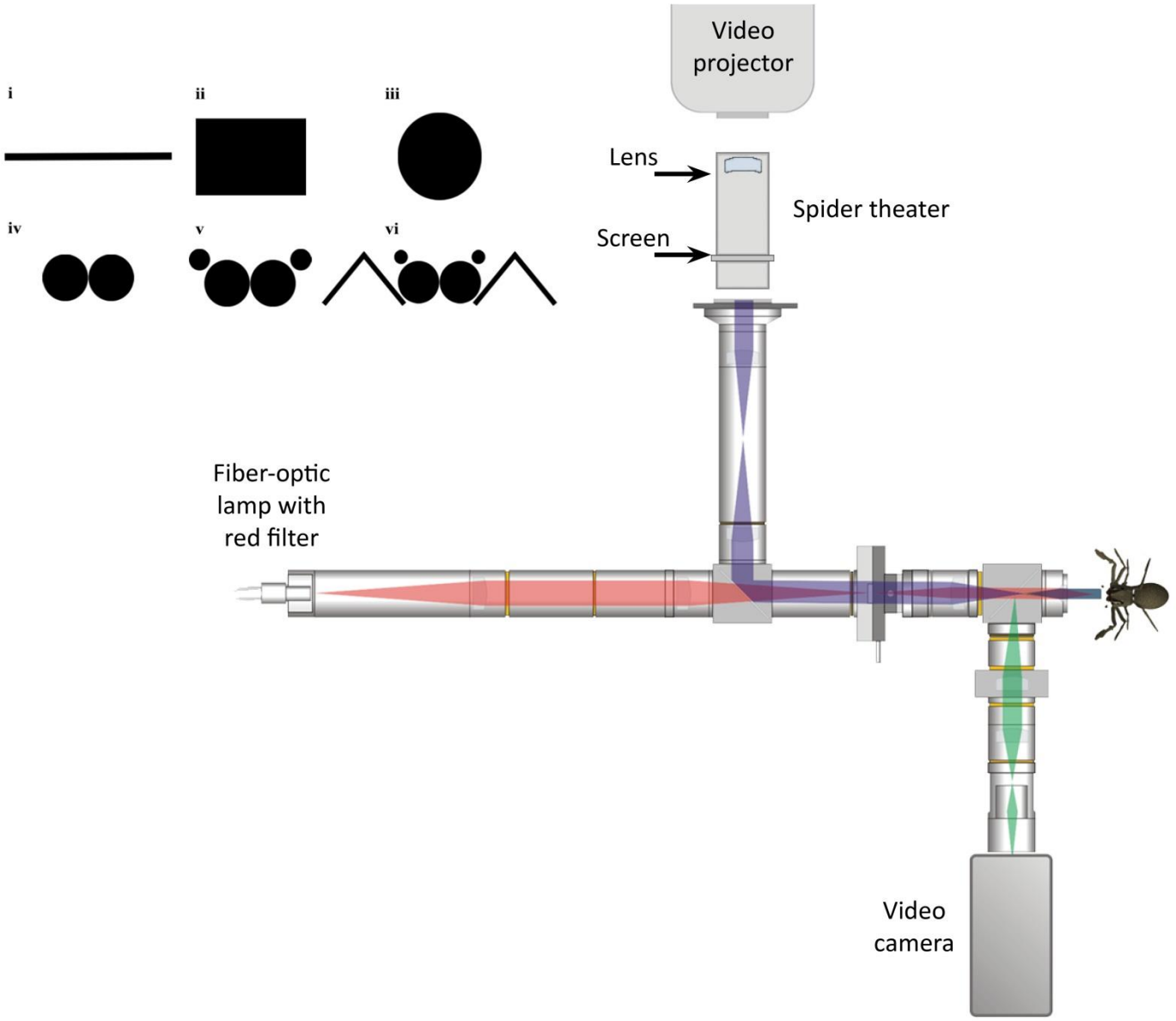

Figure S2 Eye-tracker layout depicting the different light pathways. Near infra-red light (red) is projected into the spider's eyes and reflected (green) into the video camera. A stimulus is projected (purple) through a reducing lens onto a screen which is visible to the spider. Top inset: The different stimuli used in these experiments: i to vi, respectively: a horizontal bar, a square, a circle, two circles side-by-side, four circles (two large, two small) arraged in an anterior view of the salticid eye configuration, and a stick figure image of the anterior aspect of a jumping spider.

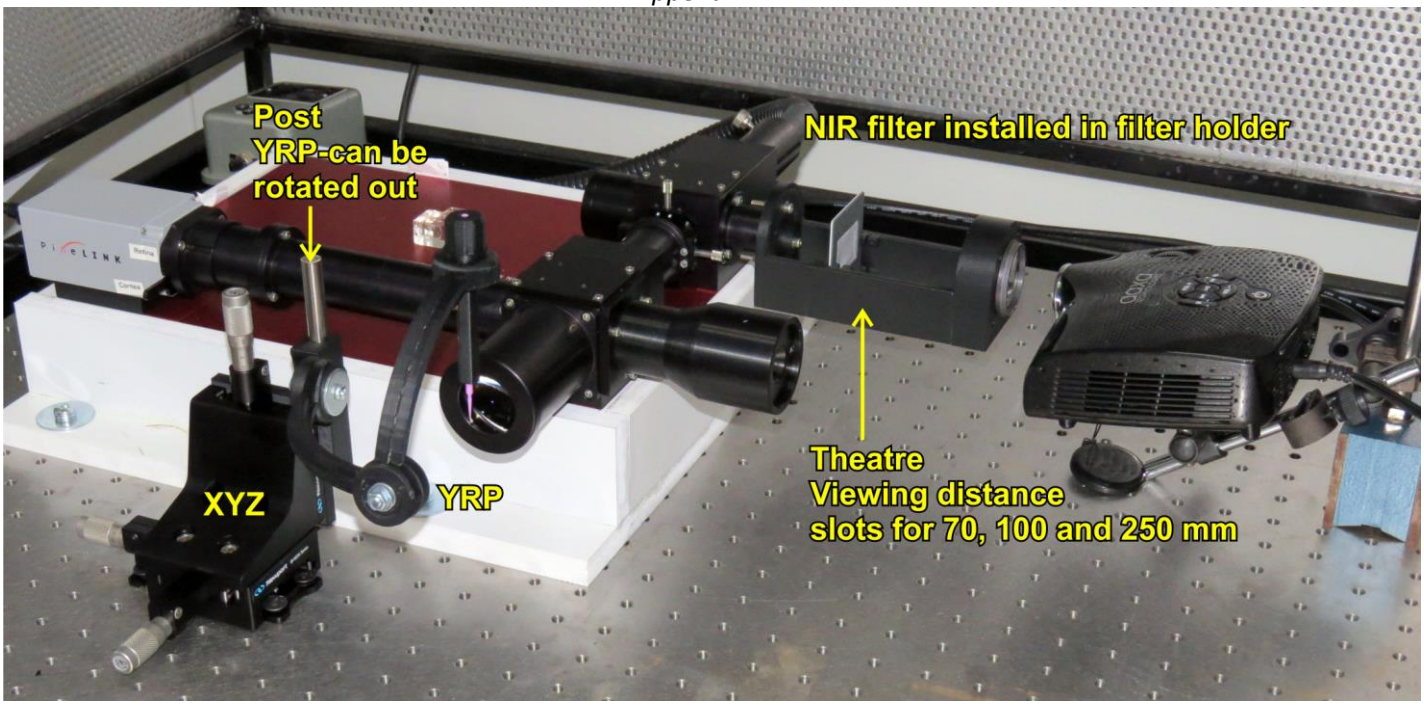

Figure S3 Picture of the setup of the eye-tracker on a vibration isolation table. Note that in this study, the projector and theater were detached from the setup and placed on a movable platform. The YRP manipulator (Figure S7) was attached to the XYZ micromanipulator by sliding it on a post, which enabled easy rotation away from the eye-tracker to place and remove the spiders easily.

Figure S4 A photo of the iris inserted in front of the IR camera.

Figure S5 Picture of one of the mask slides used in this study. On the right of the spider are the measurements of the diameter of the inner circle, the outer circle (the window) and the distance between the two circles. In this instance, inner circle radius (In) = 0.4mm; outer circle radius (Out) = 1mm; Gap between the large circles (Gap) = 0.35mm.

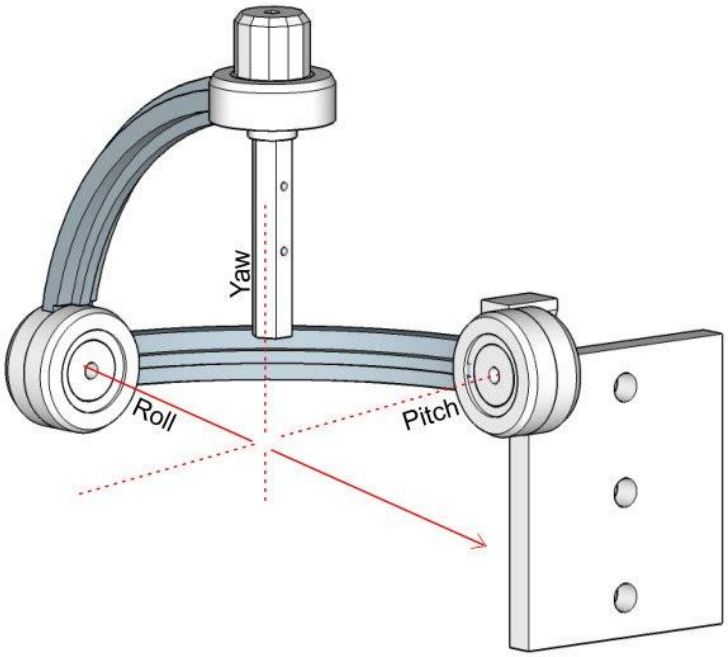

Figure S6 Illustration of the hexidimensional salticid translator (XYZ-YRP holder) which allows the alignment of the yaw, roll and pitch of the spiders. The design was created in Trimble SketchUP 2014 and printed on a UP2 3D printer.

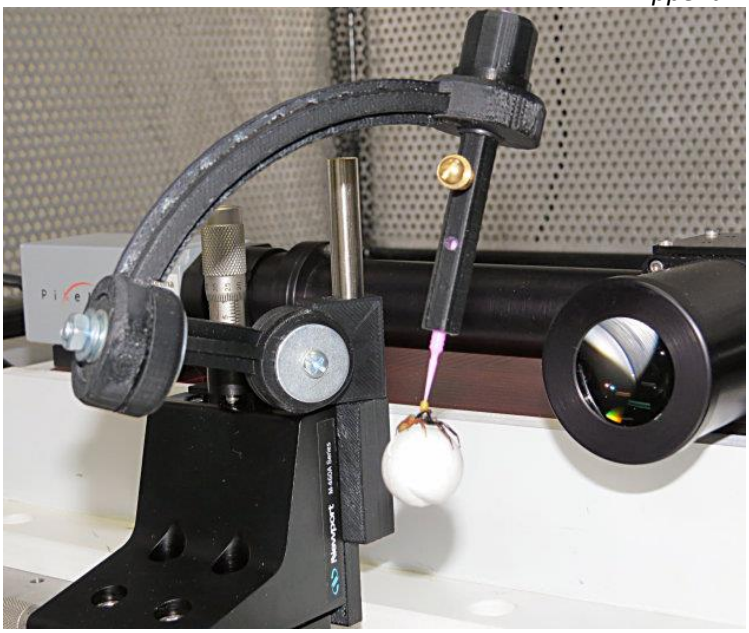

Figure S7 A male *Trite planiceps* salticid (not used in this study) in the XYZ-YRP holder in front of the eye-tracker, held in place using a dental wick dabbed in beeswax.

References

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Appendix 3

Out of its depth: A reassessment of depth judgements in jumping spiders - Supplementary information

Figure S1: Picture of the moveable platform setup with the projector (right) and theater (left) on top of it.

Axial length model

The calculations used in this model work within Geometric Optics in the paraxial approximation where we can approximate $\sin\theta \approx \theta$, where θ is the angle between the optical ray and central axis of the optical system – in this case, the spider's eye. For computational simplicity, the matrix approach was used (see Dagg and Vanderkooy 1973).

The first step is to construct a 'system' matrix for the combined two lens system of the jumping spiders which explains how to traverse all the optics involved:

$$\begin{bmatrix} a & b \\ c & d \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ \frac{n_{lens2}}{n_{tissue}} - 1 & \frac{n_{lens2}}{n_{tissue}} \\ R_{back2} & \end{bmatrix} \begin{bmatrix} 1 & \frac{d_2}{n_{lens2}} \\ 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & 0 \\ \frac{n_{cells}}{n_{lens2}} - 1 & \frac{n_{cells}}{n_{lens2}} \\ R_{front2} & \end{bmatrix} \begin{bmatrix} 1 & \frac{(L \pm \Delta L)}{n_{cells}} \\ 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & 0 \\ \frac{n_{lens1}}{n_{cells}} - 1 & \frac{n_{lens1}}{n_{cells}} \\ R_{back1} & \end{bmatrix} \begin{bmatrix} 1 & \frac{d_1}{n_{lens1}} \\ 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & 0 \\ \frac{n_{air}}{n_{lens1}} - 1 & \frac{n_{air}}{n_{lens1}} \\ R_{front1} & \end{bmatrix}$$

where *lens 1* is the corneal lens, and *lens 2* is the pit lens, n is the respective refractive index with n_{cells} the reflective index of the glass cells inside the eye tube and n_{tissue} is the refractive index of the tissue between the pit lens and the primary eye retina. *R* is the radius of curvature of the front (distal) and back (proximal) of the respective lenses, *d* the thickness of the respective lenses, and *L* is the axial length (i.e., the distance between the corneal lens and the pit lens).

The second step is to solve the imaging problem. To do that, a 'transfer' matrix is calculated in order to get from an object to a retinal image:

$$\begin{bmatrix} A & B \\ C & D \end{bmatrix} = \begin{bmatrix} 1 & \frac{S_i}{n_{tissue}} \\ 0 & 1 \end{bmatrix} \begin{bmatrix} a & b \\ c & d \end{bmatrix} \begin{bmatrix} 1 & \frac{S_0}{n_{air}} \\ 0 & 1 \end{bmatrix}$$

where S_0 is the distance from the object to the vertex of the first lens, and S_i the distance from the vertex of the pit lens. Note that these calculations assume a focussed retinal image.

The imaging condition is that element B of the transfer matrix equals zero, which is equivalent to saying that the rays leaving a fixed point on the object must meet at the same point on the image, regardless of the angle with which they depart the object. Thus, solving for B = 0 will give us the object distance (S_0) which is in focus for a given axial length (*L*). The parameters used for our modal are summarised in Table S1 below. Data were extracted from Land (1969), Williams and McIntyre (1980), and Blest et al. (1981); however, as Blest et al. (1981) stated, in many cases, the precise geometry of the lenses (pit or corneal) in not known, and some parameters are assumed.

Table S1: Optical measurements used for the model. Values were taken from Land (1969), Williams and McIntyre (1980), and Blest et al. (1981).

	Portia fimbriata		***Phidippus johnsoni***	
	Corneal lens	**Pit lens**	**Corneal lens**	**Pit lens**
Lens radius of curvature (R) (front; back)	427.689; 427.689[1]	-5;-5	344; -179	-5;-5[2]
Lens reflective index (n)	1.4	1.4	1.4	1.4[2]
Lens thickness (d)	330	235	435	235[2]
Glass cells refractive index (n)	1.336		1.336	
Tissue refractive index (n)	1.4		1.4	

[1]Exact measurement not given; value was estimated by illustration of *P. fimbriata* lens in Williams and McIntyre (1980).

[2]Assumed to be the same as in *P. fimbriata* (Williams and McIntyre 1980), as no measurements of the pit lens were found.

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Appendix 4

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Innate Pattern Recognition and Categorization in a Jumping Spider

Yinnon Dolev*, Ximena J. Nelson

School of Biological Sciences, University of Canterbury, Christchurch, New Zealand

Abstract

The East African jumping spider *Evarcha culicivora* feeds indirectly on vertebrate blood by preferentially preying upon blood-fed *Anopheles* mosquitoes, the vectors of human malaria[1], using the distinct resting posture and engorged abdomen characteristic of these specific prey as key elements for their recognition. To understand perceptual categorization of objects by these spiders, we investigated their predatory behavior toward different digital stimuli - abstract 'stick figure' representations of *Anopheles* constructed solely by known key identification elements, disarranged versions of these, as well as non-prey items and detailed images of alternative prey. We hypothesized that the abstract images representing *Anopheles* would be perceived as potential prey, and would be preferred to those of non-preferred prey. Spiders perceived the abstract stick figures of *Anopheles* specifically as their preferred prey, attacking them significantly more often than non-preferred prey, even when the comprising elements of the *Anopheles* stick figures were disarranged and disconnected from each other. However, if the relative angles between the elements of the disconnected stick figures of *Anopheles* were altered, the otherwise identical set of elements was no longer perceived as prey. These data show that *E. culicivora* is capable of making discriminations based on abstract concepts, such as the hypothetical angle formed by discontinuous elements. It is this inter-element angle rather than resting posture that is important for correct identification of *Anopheles*. Our results provide a glimpse of the underlying processes of object recognition in animals with minute brains, and suggest that these spiders use a local processing approach for object recognition, rather than a holistic or global approach. This study provides an excellent basis for a comparative analysis on feature extraction and detection by animals as diverse as bees and mammals.

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* E-mail: yinnond@gmail.com

Introduction

Object recognition is the ability to perceive the physical properties (such as shape, color and texture) of an object and apply semantic/cognitive attributes to the object [1], such as an understanding of its use, or classification of the object as prey, predator or irrelevant. The process leading to recognition is typically, though not exclusively, viewed as a bottom-up hierarchy in which information is processed sequentially with increasing complexity. In vertebrates, the idea is that lower-level cortical processors, such as the primary visual cortex, process the basic object components such as color, depth and form, while higher-level cortical processors, such as the inferotemporal cortex in humans, are ultimately responsible for recognition [2]. Historically, perhaps one of the best-known attempts at explaining perception and recognition is that of Gestalt psychology.

The central tenet of Gestalt psychology is that the whole differs from the sum of its parts. The theoretical framework underlying Gestalt ideas is holism, which states that systems and their properties should be viewed as wholes, not as collections of parts [3]. This contrasts with earlier structuralist hypotheses, which state that perceptions can be derived by identifying the elementary parts [4–6]. Modern research into visual processing has changed its focus from gestaltism vs. structuralism to global vs. local processing [6,7], with an expanded focus from the psychological processes of perception to include physiological processes [8]. The global processing framework results in the notion that an object is recognized only when its elements form the whole image, while the local processing framework requires the identification of correct elements, points and edges, but not necessarily the image as a whole. This distinction also suggests potential differences in the neurobiological processes underlying object recognition [8].

For a predator that relies on vision, visual ability to classify an object as predator or prey will be under strong selection. However, the extent to which visual predators further classify items can vary considerably. Some predators make rapid decisions and do minimal classifying of prey into particular types, relying instead on key features, such as seeing an object of a specific size range moving in a specific orientation, as identifiers of prey [9–12]. Examples of this approach can be found among amphibians [12,13] and mantises [14], which adopt remarkably similar approaches despite possessing very different nervous systems. Many jumping spiders (Salticidae) also rapidly categorize objects as prey or non-prey based on only a few key features [15–18]. However, it is also amongst the salticids that some of the most

precise prey identification and prey preference behaviors among animals is found.

An extreme case of such preference is that of *Evarcha culicivora*. Uniquely, this East African salticid feeds indirectly on vertebrate blood by selectively preying upon female mosquitoes (particularly *Anopheles*, famous as the vectors of malaria) that have recently fed on blood. These spiders are capable of using vision alone to discriminate between their preferred prey, blood-fed female *Anopheles,* and similar looking male *Anopheles*, female *Anopheles* that have not fed on blood, non-anopheline mosquitoes, as well as various similar-sized non-mosquito prey [19–21]. These experiments have also shown that for correct identification *E. culicivora* uses a complex non-linear process involving specific elements of the prey, including an engorged abdomen, resting posture and antennae [20,22].

Like other salticids, *E. culicivora* has exceptional eyesight, which is used to locate, stalk and finally pounce on its prey [23]. Salticids have large forward-facing principal eyes that are specialized for high resolution vision but within a very narrow (ca. $<5^{\circ}$) field of view [24–28] which is compensated for with complex movements that scan up to ca. 28° to either side of the body axis [27]. Additionally, salticids have three pairs of motion-sensitive secondary eyes with wide fields of view and which collectively encompass up to 360° [29–32].

E. culicivora's unique dietary preferences, which can be expressed using vision as the sole sensory modality for prey classification [20,22], make this species an excellent subject for the study of recognition and classification of prey. Here we presented the spiders with abstract representations of potential prey ('stick figures') differing in their level of simplicity to determine whether predatory behavior and prey classification was elicited by biologically unrealistic prey containing only key elements (local processing). Stimuli included stick figures of *Anopheles* mosquitoes in their resting posture, as well as non-prey items and alternative prey items. We used single-choice predatory behavior experiments to determine whether or not *E. culicivora* 'viewed' abstract representations of prey as potential prey, and two-choice predatory behavior experiments to test for specific preference between stimuli. Due to *E. culicivora*'s known ability to discern specific elements of prey, we predicted that these specialized visual hunters would stalk and pounce on abstract representations of prey. We also predicted that *E. culicivora* would choose simplified representations of its preferred prey over realistic images of alternative non-preferred prey, showing that it categorizes these images as its preferred prey item.

Results

a. Do Jumping Spiders View Abstract Images of Prey Elements as Prey?

A total of 195 successful sessions were run in the single-choice predatory behaviour experiment: 85 with adult females, 50 with adult males and 60 with juvenile spiders. When spiders initiated stalking behavior, this almost always resulted in pouncing on the abstract prey (Table S1). The type of stimulus had no effect on whether the spiders noticed it ($\chi^2 = 6.71$, df = 6, p = 0.349, Table S2). Stimulus type did affect the propensity to stalk the prey once it was noticed ($\chi^2 = 37.87$, df = 6, $p<0.001$), but did not affect the amount of time it took the spiders to 'decide' to stalk the prey (time between the spider first noticing the stimulus and initiation of stalking behavior; $\chi^2 = 3.928$, df = 6; p = 0.686, Table S2). Once stalking was initiated, stimulus type had no effect on the propensity to pounce ($\chi^2 = 4$, df = 6, p = 0.677, Table S2). We therefore considered stalking to be a true sign of predatory behavior by the spiders. The spiders stalked the abstract images of mosquitoes (stimuli 1, 2, 3 and 4) significantly more often than the images of non-prey items (stimuli 6 and 7; Table 1). However, while the image of the fly (stimulus 5) was stalked significantly more often than the altered, disarranged abstract image of the blood-fed mosquito (stimulus 7), it wasn't stalked more often than the image of the circle (stimulus 6).

GLMs on the propensity to stalk showed significant main effects of stimulus type ($\chi^2 = 22.315$, df = 6, $p<0.005$) and spider sex ($\chi^2 = 7.413$, df = 2, $p<0.05$), but not their interaction ($\chi^2 = 9.270$, df = 11, p = 0.597). The effects of the relative contrast of the stimuli or its interaction with spider sex were also not significant (respectively, $\chi^2 = 0.039$, df = 1, p = 0.843; $\chi^2 = 0.431$, df = 2, p = 0.806). Females and juveniles were more prone to stalk stimuli (56.5% of 85 and 70% of 60 respectively) than males (38% of 50; females vs male: U = 1732.5, $p<0.05$; juveniles vs males: U = 1020, $p<0.001$, Mann-Whitney U test, Data, Tables S9, S11), while there was no significant difference between females and juveniles (U = 2205, p = 0.099, Mann-Whitney U test, Data, Table S11). Similarly, stimulus type had a significant effect on the propensity of females and juveniles to stalk (respectively,:: Cochran's Q = 14.195, $p<0.05$; Cochran's Q = 14.261, $p<0.01$, Data, Tables S3, S4, S7, S8) but not on that of the males (Cochran's Q = 5.636, p = 0.465, Data, Tables S5, S6). While there were no significant differences in how often the different sexes noticed different stimuli ($\chi^2 = 5.762$, df = 2, p = 0.056, Kruskal-Wallis test, Data, Table S10), there were significant differences in the distances at which the they noticed the stimuli ($\chi^2 = 14.021$, df = 2, $p<0.005$, Kruskal-Wallis test, Data, Tables S10), with the females noticing the stimuli from significantly further away than males or juveniles (respectively, U = 1471, $p<0.005$; U = 1751, $p<0.0.005$, Mann-Whitney U tests, Data, Table S11). There were also significant differences between the sexes in their propensity to pounce once stalking was initiated ($\chi 2 = 10.461$, $p<0.01$, Kruskal-Wallis test, Data, Tables S9, S10), with the males less prone to pounce on prey than either females or juveniles (respectively: U = 306.5, $p<0.01$; U = 267, $p<0.01$, Mann-Whitney U tests, Data, Table S11). See Data, Tables S1–S11 for the full datasets.

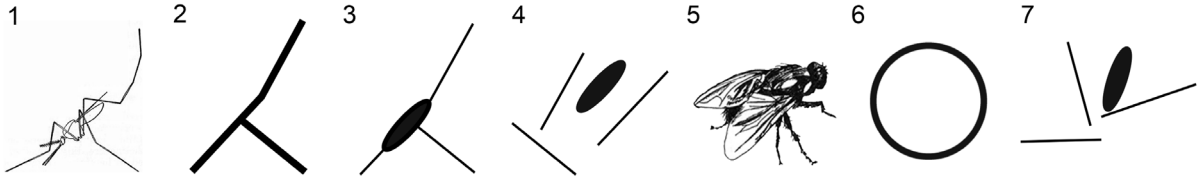

Figure 1. Images (and numbering as referred to in text) used as stimuli in both experiments. Images 1–4 are based on Anopheles mosquitoes. 1 is based on [60]. Image 4 is a disarranged version of image 3. Image 8 is a disarranged version of image 6. Image 7 is based on image 4 where the angles of the various elements have been altered.
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Table 1. Responses and statistical comparisons of the spiders to the different stimuli.

Stimulus	1	2	3	4	5	6	7
N	**27**	**28**	**27**	**30**	**29**	**27**	**27**
Stalk (%)	**74**	**64**	**74**	**77**	**56**	**33**	**24**
1	-	0.508	1	1	0.267	<0.001	<0.001
2	-	-	0.549	0.549	0.774	<0.05	<0.005
3	-	-	-	1	0.267	<0.05	<0.001
4	-	-	-	-	0.302	<0.005	<0.001
5	-	-	-	-	-	0.118	<0.05
6	-	-	-	-	-	-	0.219

Upper section of the table contains the number of spiders that noticed each stimulus (N) and the percentage of stalking instances. The bottom section of the table contains the crosswise comparisons of the stalking responses to the different stimuli, using McNemar tests with a binomial distribution. See Figure 1 for stimulus images. Note all image sizes are equivalent, see Table 3.

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b. Do Jumping Spiders View Abstract Images as their Preferred Prey?

A total of 123 successful sessions were run in the two-choice predatory behaviour experiments, 61 with females, 34 with males and 28 with juveniles. Spiders never exhibited a side bias (experiments 1 through 5, respectively: $p = 0.23$; $p = 0.83$; $p = 0.35$; $p = 0.54$; $p = 0.54$, Binomial test). When given a choice between abstract representations of their preferred prey and a realistic image of non-preferred prey (a house fly), *E. culicivora* chose the preferred prey significantly more often (experiments 1 and 2 respectively, $p<0.001$; $p<0.01$, Binomial test, Table 2). Spiders also chose a disarranged abstract representation of their preferred prey significantly more often than they chose a realistic image of non-preferred prey (experiment 3, $p<0.05$, Binomial test, Table 2), or a disarranged non-prey item (experiment 5, $p<0.05$, Binomial test, Table 2). However, spiders showed no preference when presented with an abstract representation of their preferred prey and a disarranged version of that same image (experiment 4, $p = 0.84$, Binomial test, Table 2).

Discussion

This study shows that for *E. culicivora*, discrimination and categorization can be achieved using only visual representations of the basic elements of its preferred prey. By using stick figure drawings of their preferred prey – *Anopheles* mosquitoes, we have created stimuli constructed only of key elements of their prey that have been found to be important for recognition [20,22]. As hypothesized, we have shown that not only do these spiders view these stimuli as potential prey (by initiating predatory behavior), but they also prefer these abstract images of prey to detailed images of alternative non-preferred prey. These results show that the various elements that have been found to be necessary for prey discrimination in previous studies are [20,22] also sufficient for recognition. This was the case regardless of whether or not the spiders had encountered their preferred prey before. Our controls have ruled out external cues, such as side preference, number of elements of the stimulus, and the relative contrast of the stimuli. Interestingly, the propensity to pounce was not affected by the different stimuli, and was seen in almost all cases where stalking was initiated. It would seem that the decision to pounce relies on other cues not singled out in this study, or, perhaps more likely, that pouncing is a follow-up behavior akin to a 'fixed action pattern'.

Our confidence in these results is strengthened by the behavior of the naïve juveniles in the single-choice predatory behavior experiment. When hunting *Anopheles*, but no other type of prey, juveniles of *E. culicivora* perform an innate prey-specific predatory behavior involving a detour to approach the prey from behind [33]. This detouring approach to the prey was evident in 57% of the trials involving a stimulus representing an *Anopheles* (stimuli 1–4; $N = 31$; stimulus 1 (detours/attacks): 7/11; stimulus 2: 4/9; stimulus 3: 6/10; stimulus 4: 1/1) with juveniles, but only once with the fly stimulus (stimulus 5; $N = 8$) and never with the circle stimulus (stimulus 6; $N = 3$). Despite these small sample sizes, it is apparent that they recognize the stick-figure stimuli specifically as *Anopheles* mosquitoes.

The low level categorization of the abstract stimuli into prey and non-prey items is also seen in other invertebrates such as the praying mantis, where basic features of the stimuli, including size and speed, are the main cues [14,34]. However, *E. culicivora*'s discriminations use much finer details of an image, such of the size and shape of mosquito antennae, when making decisions regarding preference [20,22], and thus require a considerably

Table 2. Results of two-choice predatory behavior experiment stimulus pairs. Note all stimulus sizes are equivalent, see Table 3.

Experiment	N	Image 1	Image 2	Chose Image 2	p
1	22	2	5	9%	<0.001
2	28	3	5	25%	<0.05
3	28	4	5	29%	<0.05
4	24	3	4	46%	0.84
5	21	4	8	19%	<0.05

Pairs of images used in the two-choice predatory behavior experiments, percentage of pounce choices for the second image, and results of Binomial tests. See Figure 1 for images.

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higher level of feature detection. The most notable instance of such discrimination in this study was the ability of the spiders to discriminate between the two disarranged stimuli in the single-choice predatory behavior experiment, where the only difference between the stimuli were the relative angles between the elements and yet one was categorized as prey, while the other was not. Nelson and Jackson [20,22] have shown that the resting posture of a mosquito is an important cue for recognition. Our findings fine-tune those conclusions by suggesting that it is not the angle of the body compared to a surface or horizon, but rather the relative angles between the body elements that is crucial for recognition.

Discrimination of orientation has been shown in honeybees (*Apis mellifera*), which can distinguish different orientations even when these are produced through illusory contours [35] and without clear edge detection [36]. Horridge [37,38] proposed that the generalization ability of the honeybee uses different parameters of an image to form local cues. These discrimination mechanisms were based on physical aspects of an image, but Avargues-Weber et al. [39,40] demonstrated that honeybees are even capable of abstract concepts such as above-below and left-right. Unlike in the bee studies, we used unlearned stimuli and untrained animals, and show that *E. culicivora* is capable of discrimination using a significantly more complex abstract concept - angles between disconnected elements.

One way of achieving such discrimination ability is by storing the 'correct' orientation of the various elements and comparing each element to stored memory. However, the spiders occasionally pounced upon the stimulus while standing on the sides or the ceiling of the starting chamber (analogous to behavior common in a natural setting, XJN pers. obs.), suggesting that orientation effects do not play a role in these decisions. While it is tempting to consider this type of object consistency in recognition to be superior to that seen in human recognition of faces (where face recognition is degraded significantly more than other objects when viewed upside-down [41–44]), there is an inherent difference between the two - faces often have a prototypical orientation, while in the spider's natural three-dimensional environment prey is often viewed from different orientations.

An alternative mechanism of achieving the discrimination ability seen in this study is by 'calculating' the relative difference of the angles and comparing that to stored angles that represent prey. While discrimination of orientation has been well studied in vertebrates and invertebrates [45–49], relative angle discrimination in non-human animals remains largely unstudied. In humans, however, this ability has been well studied (e.g., [50–52]) and there is some evidence for a neural mechanism that encodes angles in humans [53], as well as in macaques [54] and cats [55].

Our results demonstrate that *E. culicivora* not only categorizes the simplified abstract stimuli as prey, but recognizes them as its preferred prey, exhibiting higher level categorization or within-category discrimination. This was the case even for the disarranged version of the blood-fed *Anopheles*, a capability not dissimilar to that of humans with visual expertise when viewing fragmented images of cars or faces [56], although in our case the images were abstract and dispersed rather than fragmented. *E. culicivora* not showing any preference between the blood-fed *Anopheles* stimulus and its disarranged version was perhaps the most surprising finding of this study. While it is possible that *E. culicivora's* response to the image of the disarranged *Anopheles* was due to its resemblance to some other unknown prey rather than *Anopheles*, this is unlikely as the dietary preferences of these spiders has been well studied [19,20,22,33]. We should note that experiments using stimuli 4 and 7 were both run at a later date. While this too might have affected the results, this also seems unlikely, as the laboratory conditions were constant and the spiders were healthy. Another alternative explanation is that the specific arrangement of the elements of the disarranged *Anopheles* exploits a sensory bias in the *E. culicivora's* visual pathways, while the altered version of this stimulus does not. Regrettably, we could not test the spider's responses to other alternative arrangements of these stimuli. Nonetheless, either through a sensory bias in the visual pathways, or by higher level visual analysis, the spiders evidently categorized both the blood-fed *Anopheles* stimulus and its disarranged version as their preferred prey. This suggests that they do not use a global, or holistic approach to recognition [4,44], but rely instead on the analysis of specific elements at a local level to recognize an object [6–8]. This type of analysis functions much like distributed feature extraction algorithms of object recognition in computer vision based upon the vertebrate visual cortex [57,58], in which low-level areas of the nervous system are delegated to recognizing different elements which are then fed to higher order centers [59]. A closer look at how these spiders visually analyze what it is they are seeing will provide a deeper understanding of what specific features these spiders are looking for when they are looking for prey.

Methods

a. General

All spiders used in this study were at least second generation laboratory reared individuals, and no juveniles tested had ever encountered mosquitos. Testing was carried between 0730 and 1200 h in a ÿharacteriz-controlled laboratory set to 24° with a photoperiod of 12L:12D, lights on at 07:00. Test spiders were unmated adults (body length, 4.5–5.5 mm) and juveniles (1.5–2.5 mm). Standard rearing and maintenance was as in earlier studies (for details, see [19,20]). Spiders were caged individually and were fed to satiation once a week on *Drosophila* spp. Two h

prior to their use as prey, *Drosophila* were given a honey and human blood (obtained from a blood bank) meal by inserting a cotton dental wick dipped in the mixture into their rearing container. Test spider hunger levels were standardized by a 5–7 day pretrial fast. Test spider predatory behaviors (noticing, stalking and/or pouncing) and their timing were recorded during all experiments. Noticing ÿharacte is ÿharacterized by the spider performing an optomotor response to face the stimulus with its AM eyes and subsequently staring continuously at the stimulus for a few seconds. Stalking behavior is ÿharacterized by the salticid slowly stepping toward the prey while visually fixated on the prey. Both are reliably identifiable behaviors.

b. Stimuli

Stimuli consisted of videos of repeated sporadic movement of different images (Figure 1, Table 3), created using Adobe Photoshop CS5 in greyscale. Image 1 was a realistic line drawing of a blood-fed female *Anopheles gambiae* mosquito in typical resting posture, while the simplified images 2 (not blood-fed) and 3 (blood-fed) were similar but used only straight lines and ovals, with the latter depicting a blood-fed mosquito with an engorged abdomen, known as an important prey-identification cue [22]. Image 4 was a disarranged version of image 3, created so as to not alter the respective angles of any of the elements of image 3, while ensuring the elements were disconnected and, to humans, no longer resembling a mosquito. Image 5 was created by rendering a photograph of a housefly (*Musca domestica*) to grayscale and removing the background. Image 6, a circle the size of a housefly was created as a control, as were images 7 and 8. Image 7 was an altered version of image 4 where the angles of each of the elements of the image were altered and image 8 was a disarranged version of image 6, broken into 4 unequal sections. All images were created on a background of 250, 250, 250 RGB and had black pixel counts between ca. 200 and 550 pixels (Table 3). Screen size was set to 1024×768 pixels. All images were sized similarly and were presented at biologically relevant sizes (to the nearest 0.5 mm).

To create the stimuli, one (in single-choice predatory behavior experiments) or two (in two-choice predatory behavior experiments) images were rendered into videos of repeated, horizontal (single-choice predatory behavior experiments) or vertical (two-choice predatory behavior experiments) motion (two bouts of back and forth movement every 10 s). Motion speed was 9°/s, at a viewing distance of 10 cm, and movement distance was set to be 8° visual angle. These parameters were selected to maximize the attention of the spiders [31,32] (see Video S1 for a sample stimulus video).

Videos were projected onto a screen using an AAXA M2 Micro Projector connected to a computer, and placed 100 mm from the screen. The videos were played on a continuous loop using VLC player software. The screen was made of two protective sheets of glass (each 2 mm thick, 5 cm wide×5 cm long) with LCD screen polarizers from a Toshiba Tecra A9 PTS52C-MH409C laptop cut to size between them. This setup was used as we have found that the screen polarizers effectively reduce the brightness of the projected videos and did not result in a polarized image, while the glass sheets prevented the screen polarizers from getting damaged while being handled and cleaned. Due to the high spatial resolution of salticid principal eyes (ca. 11 minutes of arc, [25]) images projected directly onto a screen will appear pixelated once the spider gets close. To overcome this, while maintaining life size images at high resolution, larger than life size stimuli were back-projected through a lens placed between the projector and a screen, which reduced the projected image by ca. a factor of 10.

Table 3. Parameters of the images used in the stimuli of both experiments. Relative contrast is the number of black pixels in the frame.

Image	1	2	3	4	5	6	7	8
Relative Contrast	250	230	518	545	500	211	545	211
Width (mm)	7	6	6	6	7	6	7	7

For the actual images used see Figure 1.
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Fine tuning the size of the projected stimuli was achieved by varying the size of the VLC player window on the computer monitor.

c. Do Jumping Spiders View Abstract Images of Prey Elements as Prey?

To answer this question, we tested the predatory responses of the spiders to individual stimuli (single-choice predatory behavior experiment). An angled wooden ramp supported by a wooden pole glued to a wooden base was placed in front of the screen and projector (see Figure 2a for dimensions). The apparatus was painted with two coats of polyurethane, but the top face of the ramp had a sticker marked with a 5 mm grid to allow accurate measurement of the spider's distance from the stimulus when a particular behavior was observed. The ramp was wiped with 80% ethanol and allowed to dry for 15 min between each test to eliminate possible chemotactile traces from spiders in previous tests.

For each test, a spider was placed on the ramp and covered with a petri dish, at a distance of 6 (juveniles) or 10 (adults) cm from the center of the petri dish to the screen. These distances were used as they are far enough from the screen so that the spiders couldn't 'wald' directly onto the stimuli, while being close enough to enhance the chances of the spiders reacting to the stimuli (juveniles were less responsive to stimuli at a distance compared to adults). The screen was covered with a piece of black cardboard until test spiders were released to prevent them seeing the stimulus until tests began. Once the spiders were relaxed (staying stationary or grooming) the screen was uncovered, the petri dish was removed and timing started. Tests ended when the spiders pounced on an image or walked/jumped off the ramp. If a spider noticed the image, the session was considered successful and tests were not repeated with the same spider. A spider that failed to notice the stimulus was tested up to twice in one day, or up to a total of 4 times in the following 3 days.

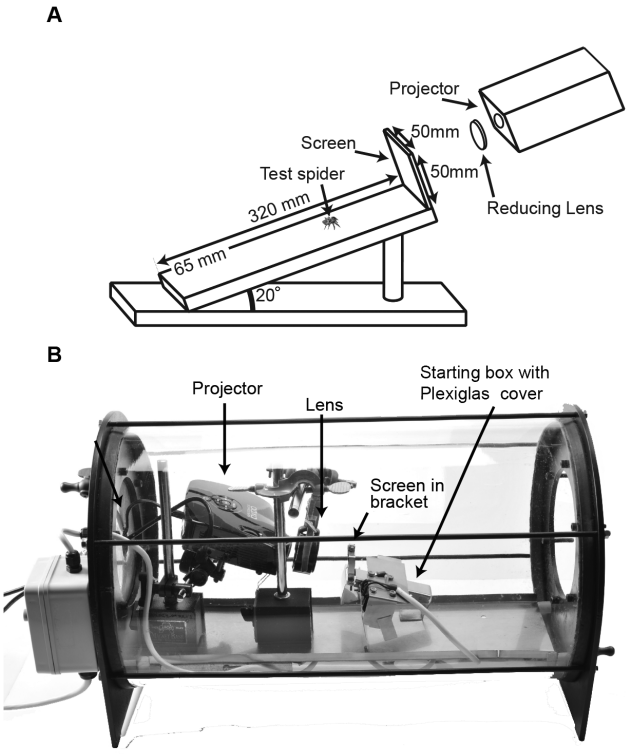

Figure 2. Experimental apparatuses used. a) Apparatus used in single-choice predatory behavior experiment. Spiders (not to scale) were placed either 10 cm (adults) or 6 cm (juveniles) away from stimulus screen, and behavior recorded. b) Apparatus used in the two-choice predatory behavior experiment. Projector and reducing lens placed inside glass chamber 100 mm from screen and ramp complex.
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d. Do Jumping Spiders View Abstract Images as their Preferred Prey?

In this experiment we relied on *E. culicivora*'s proven preference for *Anopheles* mosquitoes and presented them with a two-choice test. All spiders used in this test were laboratory reared and had no prior experience with mosquitoes. For these tests, rendered movies contained two images (Table 1) which moved identically and simultaneously. In each test, which image was on the right and which was on the left was randomized. The movies were projected as above, but experiments were held within a specialized apparatus containing a stainless steel ramp (15 mm wide×150 mm long; angled up by 25°) in front of the screen. The ramp was inside a glass chamber (diameter 300 mm, length 525 mm long) with removable sealing steel end plates (diameter 200 mm, kept off during this set of experiments). Welded to the ramp was a bracket onto which the screen was attached with a gap of 5 mm from the ramp. The ramp/screen unit ('ramp complex') sat mounted within holes on a stainless steel platform spanning the length of the cylinder (Figure 2b). In this way it could be removed for cleaning with 80% ethanol after each test and returned to the same place, while ensuring that the distance between the screen and the reducing lens and projector was always the same (and thus stimulus size was constant).

At a distance of 22 mm from the end of the ramp, a stainless steel 'starting box' (11 mm wide×19 mm high×22 mm deep; i.e., furthest point 44 mm from top end of ramp) was welded to the ramp complex (Figure 2b). The box had a transparent Plexiglas cover wired to an external controller so that it could be opened remotely. The spider was placed into the starting box and the door was closed. After 2 min, the ramp complex was put in place. Once the spider was away from the door of the starting box, after ca. 20 s, the door was opened and tests began. Tests ended with the spider pouncing on one of the two images on the screen or to jumping/walking off the ramp. Failing these two conditions, tests were stopped after 15 min. In this experiment we were interested in pouncing ÿehavior rather than installking ÿehavior, as the former constitutes a more distinct choice by the spiders. For this reason, both adult and juvenile spiders were released a short distance from the screen (see Video S2 for a sample of the spider behavior in this experiment).

e. Data Analysis

All analyses were done using SPSS Statistics v.20. For the single-choice predatory behavior experiment, GLM analyses were performed to check for the main effects of stimulus type, 'sex', relative contrast (number of black pixels against a white background) which was either in the ca. 200 or ca. 500 pixels) and their interaction on the spider's choice to stalk the stimuli. Interactions between stimulus relative contrast and stimulus type were not analyzed, as these are nested. Sexes were divided into three – female, male and juvenile as their sex cannot be discerned and their behavior differs [22,33]. In this model the dispersion parameter was set at 1, and type III sums of squares were used, though there was no qualitative difference from type I. Kruskal-Wallis tests were used to compare the predatory responses between the different sexes, with Mann-Whitney U tests for pairwise

analysis. Cochran's Q tests were used to test how the different stimuli affected the chances of the spiders noticing the stimulus and the propensity to stalk and pounce. Friedman tests were used to test the effects of the different stimuli on stalking initiation distance, as well as their effects on the amount of time it took the spiders to start stalking. When these effects were found to be significant, McNemar tests were used for pairwise comparisons. For the two-choice predatory behavior experiments, Binomial tests were used to test the spider's choices, as well as possible side-bias.

Supporting Information

Table S1 Results from the single-choice predatory behavior experiment (all spiders). M = Median, IQR = interquartile range. The percentages of the spiders that stalked/pounced are nested within the percent of spiders that noticed/stalked, respectively. See Figure 1 for stimulus images.
(DOC)

Table S2 Statistics comparing between the different stimuli for the single-choice predatory behavior experiment (results from all spiders; data in Table S1). *Cochran's Q; **Friedman's test (χ^2); df = 6 in all tests.
(DOC)

Table S3 Results from the single-choice predatory behavior experiment (female spiders). M = Median, IQR = interquartile range. The percentages of the spiders that stalked/pounced are nested within the percent of spiders that noticed/stalked respectively. See Figure 1 for stimulus images.
(DOC)

Table S4 Statistics comparing between the different stimuli for the single-choice predatory behavior experiment (results from female spiders; data in Table S3). *Cochran's Q; **Friedman's test (χ^2); df = 6 in all tests.
(DOC)

Table S5 Results from the single-choice predatory behavior experiment (male spiders). M = Median, IQR = interquartile range. The percentages of the spiders that stalked/pounced are nested within the percent of spiders that noticed/stalked, respectively. *Insufficient cases for IQR. See Figure 1 for stimulus images.
(DOC)

Table S6 Statistics comparing between the different stimuli for the single-choice predatory behavior experiment (results from male spiders; data in Table S5). *Cochran's Q test; **Friedman's test, $\chi 2$; ***Insufficient cases for analysis; in all tests, df = 6.
(DOC)

Table S7 Results from the single-choice predatory behavior experiment (juvenile spiders). M = Median, IQR = interquartile range. The percentages of the spiders that Stalked/Pounced are nested within the percent of spiders that Noticed/Stalked respectively. *Insufficient cases for IQR. **No juveniles tested with this stimulus. See Figure 1 for stimulus images.
(DOC)

Table S8 Statistics comparing between the different stimuli for the single-choice predatory behavior experiment (results from juvenile spiders; data in Table S7). *Cochran's Q test; **Friedman's test (χ^2); in all tests, df = 5.
(DOC)

Table S9 Results from the single-choice predatory behavior experiment for each sex/age group. M = Median, IQR = interquartile range, F = female, M = male, Juv = juvenile. The percentages of the spiders that stalked/pounced are nested within the percent of spiders that noticed/stalked, respectively.
(DOC)

Table S10 Statistics comparing between different sex/age groups for all stimuli in the single-choice predatory behavior experiment; data in Table S9. Kruskal-Wallis tests (df = 2). In pairwise analysis females noticed the stimuli from significantly further away than other groups (Table S11).
(DOC)

Table S11 Differences between the sex/age groups in notice distance, stalking propensity and pouncing propensity for all stimuli in the single-choice predatory behavior experiment (data in Table S9). Pairwise analysis of differences in noticing distance, stalking propensities and pouncing propensities once stalking was initiated. Mann-Whitney U tests. F = female, M = male, J = juvenile.
(DOC)

Video S1 Sample stimulus video for the two-choice predatory behavior experiment presenting images 3 and 4.
(AVI)

Video S2 Sample session video from the two-choice predatory behavior experiment.
(MP4)

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Author Contributions

Conceived and designed the experiments: XN YD. Performed the experiments: YD. Analyzed the data: XN YD. Wrote the paper: XN YD.

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Appendix 5

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Y Dolev & XJ Nelson

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RESEARCH ARTICLE

Biological relevance affects object recognition in jumping spiders

Y Dolev and XJ Nelson

School of Biological Sciences, University of Canterbury, Christchurch, New Zealand

ABSTRACT

We investigated whether biological relevance affects the perceptual processes underlying prey classification in jumping spiders (Salticidae). We used choice experiments with abstract and realistic representations of prey to test whether *Hypoblemum albovittatum*, a generalist predator, differs in how it classifies prey compared with *Evarcha culicivora*, which specialises in preying on blood-fed *Anopheles* mosquitoes. Unlike *E. culicivora*, *H. albovittatum* preferentially chose realistic over abstract representations of prey. Both species had similar decision times when choosing realistic images, which for *H. albovittatum* was similar to its decision time with abstract stimuli. In contrast, *E. culicivora* was significantly faster at making a decision with abstract images of *Anopheles*. These results suggest that *E. culicivora* uses key feature extraction methods when confronted with its preferred prey, but otherwise relies on holistic processing of an object, which appears to be the mechanism used by *H. albovittatum*.

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Introduction

For any predator, a necessary prerequisite to prey selection is object recognition and categorisation, yet how animals achieve this fundamental task is surprisingly understudied. Visual object recognition is the ability to perceive the physical properties of an object (such as shape, colour and texture) followed by applying semantic attributes to the object (Enns 2004), such as the classification of the object as prey, predator or irrelevant. The diverse natural histories of visual predators have led to vastly different processes of classification of prey items. Some predators make rapid decisions and do minimal classifying of prey into particular types, instead relying on basic key features of an object as identifiers of prey. The use of key attributes is often used by amphibians (Barlow 1953; Lettvin et al. 1959; Heinze et al. 1998), mantises (Prete et al. 2011) and even birds (Bond 2007) to classify an object as prey. These elements include a specific size range, movement in a specific orientation, colour or pattern. In contrast, while little work has been done on visual discrimination abilities in predator–prey interactions, it is well known that many animals, including many invertebrates, are capable of learning complex visual discrimination tasks (Srinivasan 1994; Gierszewski et al. 2013; Fuss et al.

CONTACT Ximena Nelson ximena.nelson@canterbury.ac.nz

2014). As exemplified by the formation of search images, this ability can be used when hunting (Bond 2007). However, whether closely related animals categorise the same stimulus differently, or even possibly attribute different valence to it, has not been directly explored. Nonetheless, this might be expected when considering predators with different predatory behaviours and preferences.

Predators are commonly divided into generalists that consume a wide range of different types of prey or specialists which tend to consume specific types of prey more often than might be expected given their prevalence in the habitat. Predatory specialists therefore target specific types of prey, and express a preference for those particular prey types when given a choice. Perhaps one of the most interesting questions relating to prey preference is its evolution (Tauber et al. 1993; Pekár 2004; Pekár & Toft 2014) and the parallel evolution of the processes underlying object recognition and categorisation. This is because, crucially, the behavioural traits of prey preference and dietary specialisation rely on a predator's ability to distinguish between different types of prey. Consequently, a comparative approach between specialists and generalists within the same animal grouping is a powerful way to investigate both the perceptual processes underlying object classification, and the salience of different objects to specific animals.

Spiders are generally envisaged as generalists (Bristowe 1941; Wise 1993; Foelix 1996; Wise 2006), yet it is within this group, particularly among jumping spiders (Salticidae), that we find some of the most extreme cases of prey specialisation known. These include spider-eating species (Jackson & Hallas 1986; Jackson 1992; Harland & Jackson 2000, 2006), ant-eating species (Edwards et al. 1974; Cutler 1980; Jackson & Li 2001; Jackson & Nelson 2012), and even a species (*Evarcha culicivora* Wesolowska and Jackson, 2003) that has a particular preference for blood-fed female mosquitoes in the genus *Anopheles* (Wesolowska & Jackson 2003; Jackson et al. 2005; Nelson & Jackson 2006). This East African spider is capable of using vision alone to discriminate between its preferred prey and similar-looking male *Anopheles*, female *Anopheles* that have not fed on blood, non-anopheline mosquitoes, as well as various similar-sized non-mosquito prey (Jackson et al. 2005; Nelson & Jackson 2006, 2012). In contrast, most salticids are generalists, showing no preference for specific prey when given the choice between different prey types. One such salticid is the New Zealand house hopper, *Hypoblemum albovittatum* (Keyserling, 1882). While little information is available on the natural diet of *H. albovittatum*, personal observations, as well as many years of experience in the laboratory (Tarsitano & Jackson 1992), lead us to conclude that this is a generalist species.

Salticids are especially suited for investigation into visual processing and object categorisation because they are highly visual animals that respond readily to digital images on screens. Moreover, salticids are capable of discriminating minute details in a visual scene (Nelson 2010; Nelson & Jackson 2012). This is enabled by a pair of large forward-facing eyes (the anterior median or principal eyes), which are specialised for high resolution vision (spatial acuity), but within a very narrow (c. 2–5°) field of view (Land 1969a; Williams & McIntyre 1980; Land 1985; Blest et al. 1990). However, this narrow field of view is compensated for with complex retinal movements that scan up to c. 28° on either side of the body axis (Land 1969b). A tiered retina sits at the end of an elongated eye tube attached to the corneal lens, which is part of the exoskeleton. The eye tube is surrounded by six muscles, which enable horizontal, vertical and rotational movement even though the corneal lens is static (Land 1969b). In addition, salticids have three pairs of

smaller, immobile lateral eyes with a combined visual field of c. 360°. These 'secondary eyes' function primarily as motion detectors (Land 1971, 1972; Zurek et al. 2010; Zurek & Nelson 2012).

We have previously shown that the mosquito-eating salticid *E. culicivora* not only categorises abstract stick figure representations of a mosquito as prey, but also recognises stick figure *Anopheles* mosquitoes as its preferred prey, even when the comprising elements of the *Anopheles* stick figure are disarranged and disconnected from each other (Dolev & Nelson 2014). Our work on *E. culicivora* demonstrated that this species primarily uses feature extraction methods for recognising at least this kind of prey, without the need of holistic processing.

Here we test whether biological relevance affects the recognition and classification of abstract images of prey in two related predators. Specifically, we predicted that the predatory specialist salticid *E. culicivora* would differ in its ability to classify prey compared with *H. albovittatum*, a generalist salticid hunter, in accordance with the biological significance of the prey to the spider. We tested the prey choice behaviour of *H. albovittatum* in a multi-choice experiment using common prey items from the natural environment of this species, predicting that there would be no preference for any food item. We then used a two-choice test using abstract and realistic images to examine the responses of *H. albovittatum* to abstract images of *E. culicivora*'s preferred prey item—*Anopheles* mosquitoes. For this experiment we predicted that, unlike *E. culicivora*, *H. albovittatum* would preferentially choose realistic stimuli over abstract representations of prey. To test the effects of biological relevance we compared these results with our previous results with *E. culicivora* (Dolev & Nelson 2014).

Materials and methods

General

All testing was carried out between 0800 and 1400 h in a temperature-controlled laboratory set to 24 °C, with a photoperiod of 12L:12D (lights on at 0700 h). Spiders were housed individually in 1L plastic cages with a damp cotton wick for humidity. Spiders were fed to satiation once a week on *Drosophila* spp.; but before testing spiders were subjected to a 5–10 day fast. Test spiders were adult (body length, 4.5–5.5 mm) and juvenile (1.5–2.5 mm) *H. albovittatum*, a locally common and readily identifiable species. Females have a distinct abdominal pattern and a dark spot on the anterior dorsal part of their cephalothoraces, while males have dark legs and an orange band around their eyes (clypeus). All spiders were collected from houses and gardens around the University of Canterbury, and were kept in the lab for a minimum of 2 weeks before use. Gravid females were not tested.

All stimulus images used for tests (Figure 1) were created using Adobe Photoshop CS5. Figure 1A and 1C–F (from Crowe 2002) were rendered in black and white and were placed on a transparent background. Figure 1B, a circle approximately the size of a housefly, and Figure 1G, a photograph of an *Anopheles gambiae* Giles, 1902 mosquito in its typical resting posture, were also rendered in black and white with the background removed. Figure 1H was a stick figure representation of an *A. gambiae* in its typical resting posture (ensuring that the angles between all body parts were maintained) and Figure 1I was a scrambled disconnected version of Figure 1H, created so as to not alter the respective angles of any of the

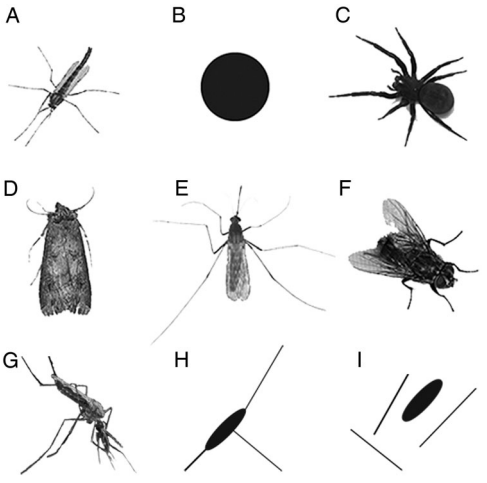


Figure 1. Stimuli used in the multiple prey choice experiment with *Hypoblemum albovittatum*. **A,** *Chironomus zealandicus* (midge); **B,** circle; **C,** *Badumna longinqua* (spider); **D,** *Eudonia angustea* (moth); **E,** *Culex pervigilans* (mosquito); **F,** *Musca domestica* (fly); **G,** *Anopheles gambiae* (mosquito); **H,** *Anopheles gambiae* (stick figure); **I,** disarranged *Anopheles gambiae* (stick figure).

elements of Figure 1H, while ensuring the elements were disconnected and, to humans, no longer resembling a mosquito.

Multi-choice experiment

These tests took place in an arena where six stimuli (Figure 1A–F) were visible to the test spider. The arena was made from 5 mm PVC sheets and was created by placing six inclined (22°) ramps around a central hexagon (the 'starting platform') placed atop a PVC base (dimensions in Figure 2). Attached at the top end of each ramp was an electric stimulus mount built using a deconstructed analogue voltmeter, which was placed in front of a white background. The needles of the voltmeters were used to mount the different stimuli by gluing a thin tube to the back of each picture and then sliding the tubes over the needles. All voltmeters were connected to a control unit to trigger stimulus movement. Each trigger consisted of recurrent electric pulses, whose frequency, amplitude and duration could be controlled, causing the voltmeter needles to simultaneously 'jiggle' 15° to each side of the vertical for 2 s (at 5 Hz). These settings were designed to be most noticeable by the spiders, as determined by preliminary experiments.

The stimuli were printed life-size on standard photopaper and cut to size. Before each test, we randomised the location of each stimulus within the arena and then the spider was placed on the starting platform at the centre of the arena (under a Petri dish) and was left to calm down for about 4 min. During this time, and throughout the test, the images were jiggled once every 30 s. Tests began when the spider was released.

We recorded every time the spiders noticed and stalked a stimulus. Noticing behaviour is characterised by the spider performing an optomotor response to face the stimulus with its anterior median eyes and subsequently staring continuously at the stimulus for several seconds. Stalking behaviour is characterised by the salticid slowly stepping towards the

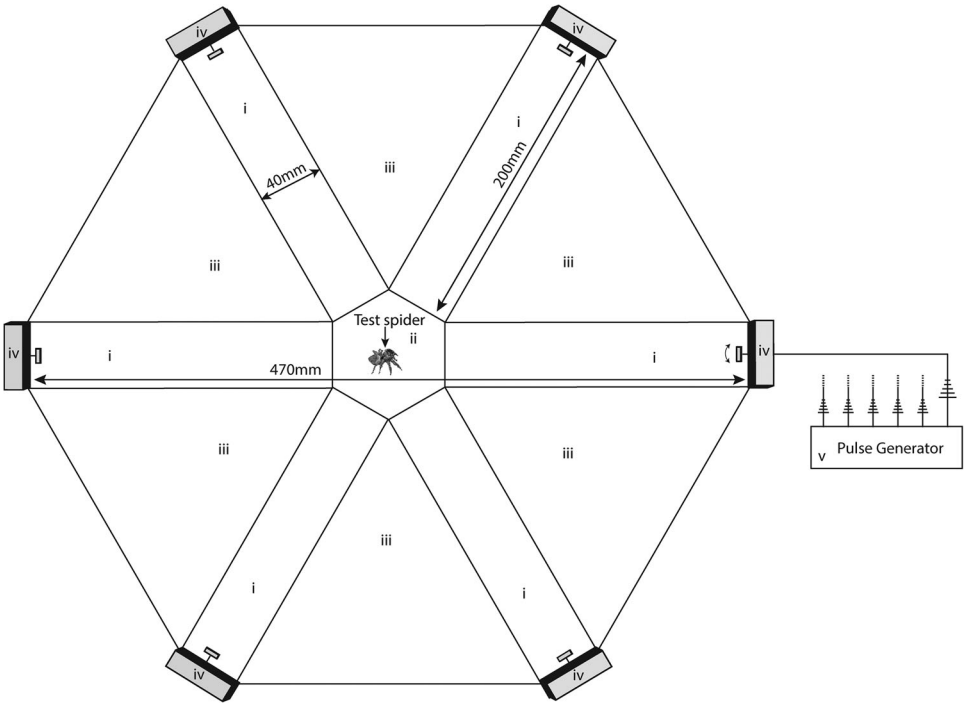


Figure 2. Test apparatus used for multi-choice experiment for *Hypoblemum albovittatum*. Roman numerals are as follows: i, angled PVC ramps (×6); ii, starting platform; iii, areas between ramps; iv, holders on which stimuli were placed at the end of each ramp; v, pulse generator connected to each stimulus holder.

prey with its body lowered while visually fixated on the prey. Both are reliably identifiable behaviours commonly used in spider behaviour experiments (e.g. Nelson & Jackson 2012; Dolev & Nelson 2014). For this experiment we regarded stalking of a stimulus as the spider making a choice. Sessions ended when the spider started stalking a stimulus, walked off the arena, or when 15 min had elapsed without the spider making a choice (the latter two were considered 'failed tests' and were used for analyses concerning attrition rate, see below).

Two-choice test

For detailed methods see Dolev & Nelson (2014). Stimuli consisted of videos containing two stimuli (Figure 1F–I), which moved identically and simultaneously. Some of these stimuli were realistic depictions of potential prey (Figure 1F–G), while others were stick figure representations of prey (Figure 1H–I), to which *E. culicivora* responds in the same manner as realistic images (Dolev & Nelson 2014). Videos were back-projected onto a frosted glass screen through a lens placed 10 mm from the projector. Stimulus motion (two bouts of movement every 10 s) was at a speed of 16°/s and moved up and down at 8° visual angle from the starting position at which *H. albovittatum* were placed. These parameters were selected to maximise the attention of the spiders (Zurek et al. 2010).

Experiments were held within an apparatus containing a stainless steel ramp (15 mm wide × 150 mm long; angled up by 25°) in front of the screen. At a distance of 22 mm from the end of the ramp, a stainless steel 'starting box' (11 mm wide × 19 mm high × 22 mm deep; i.e. furthest point 44 mm from top end of ramp) was welded to the ramp complex. The box had a transparent Perspex 'door' wired to an external controller for remote opening. Before each session, which image was on the right and which was on the left was randomised. The spider was placed into the starting box and the door was closed for about 4 min as a calming period, after which, once the spider was away from the door of the starting box, the door was opened and tests began. Tests ended with the spider either pouncing on one of the two images on the screen or jumping/walking off the ramp. Failing these two conditions, tests were stopped after 15 min. Due to the short distance between the screen and the starting position on the apparatus, the spiders were able to pounce without stalking, so only pouncing behaviour was recorded.

Statistics

All analyses were done using SPSS Statistics v.20, GraphPad Prism v.6 and R v.3.0.1. For the multi-choice experiments, χ^2 tests were performed on the spiders' choices of stimuli as well as power analyses using a medium ($w = 0.3$; see Cohen 1988) effect size. To determine any compass orientation bias, the orientation of the ramp chosen in multi-choice tests was also analysed (χ^2 tests). Spiders were divided into three groupings: female, male and juvenile (juvenile sex cannot be discerned). We used Kruskal-Wallis tests to investigate the effects of the sex or age on both prey choice and decision time (defined as the time elapsed between first noticing a stimulus and initiating stalking behaviour) in both the two-choice and the multi-choice experiments.

We used binomial tests to analyse prey choice in the two-choice experiments, and Bonferroni-adjusted χ^2 tests and Fisher's exact tests to analyse attrition rates. To analyse the decision time within and between each two-choice experiment, we used t-tests and, where appropriate, Mann-Whitney tests. These tests were also used to analyse data from our previous work on *E. culicivora* (Dolev & Nelson 2014), enabling us to directly compare the decision times and attrition rates for a specialist and for a generalist predator when viewing the same stimuli.

Results

In multi-choice tests, 41 (19 females, 15 males and seven juveniles) out of 123 sessions (33%) ended with the spiders stalking (choosing) an image. The spiders showed no significant preference for any of the six images ($\chi^2 = 1.585$, d.f. = 5, NS, $w = 0.3$, power = 0.98; Table 1) or the directionality of any of the ramps (ramps 1–6 respectively: $n = 4$, 8, 11, 9, 4, 5; $\chi^2 = 6.268$, d.f. = 5, NS). The sex or age of the spiders also had no significant effect on prey choice ($H = 1.355$, d.f. = 2, NS; Table 1). Finally, there were no significant differences in the decision time between the different choices ($H = 4.456$, d.f. = 5, NS; Table 1).

In the two-choice experiments, *H. albovittatum* showed a significant preference for the detailed 'realistic' images over the abstract images (Table 2, experiments B and D). However, they showed no preference between the two realistic images used (house fly

Table 1. Results from multi-choice prey tests for *Hypoblemum albovittatum*.

	Circle	Fly	Midge	Mosquito	Moth	Spider
Selected *n* (%)	4 (9.8)	8 (19.5)	7 (17.1)	7 (17.1)	8 (19.5)	7 (17.1)
Mean dt ± SEM	250 ± 114	489 ± 126	213 ± 39	244 ± 71	228 ± 101	188 ± 42
n for F; M; J	3; 0; 1	4; 4; 0	2; 1; 3	3; 2; 2	4; 3; 1	3; 4; 0

dt, decision time(s); F, female; J, juvenile; M, male.

and mosquito) or the two abstract ones (stick figure mosquito and its disarranged version). There were significant differences in the attrition rates between the different choice tests (2×5 test, $\chi^2 = 19.7$, d.f. = 4, $P < 0.001$; Table 2), with the attrition rate in experiment A—which consisted of two abstract images—being significantly higher than in any other experiment (Fisher's exact test with Bonferroni adjustments, experiments A vs. B: $P = 0.003$; A vs. C: $P = 0.002$; A *vs*. D: $P = 0.0007$; A vs. E: $P = 0.0015$). There were no significant differences between any of the other pairwise comparisons. No differences were found when comparing the decision time between the experiments ($H = 5.998$, d.f. = 4, NS; Table 2). Within each two-choice experiment, there were no significant differences in the decision time between the choices (experiments A–E [all NS], respectively: $t = 1.046$, d.f. = 19; $t = -1.223$, d.f. = 25, $P = 0.233$; $t = -1.037$, d.f. = 28; $t = 0.001$, d.f. = 27; $t = 0.257$, d.f. = 21; Table 2).

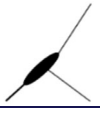
Using unpublished data (Table 3) from our previous two-choice experiments on *E. culicivora* (Dolev & Nelson 2014) we found significant differences in the decision times between the choices within experiment G ($U = 15.0$, d.f. = 2, $P = 0.001$) with the decision time for the abstract image representing a mosquito being shorter than for the realistic image of a fly (Figure 1F–I). This was also the case for experiment H, although the sample size was too small for statistical analysis due to the extreme preference for

Table 2. Stimuli used and attack rate of *Hypoblemum albovittatum* in two-choice tests.

Experiment	Image 1	Pounced on image 1 (mean dt ± SEM)	Image 2	Pounced on image 2 (mean dt ± SEM)	*P*	*n* (mean dt ± SEM)	Attrition (%)
A		12 (387 ± 40)		9 (323 ± 47)	0.66	63 (360 ± 31)	67
B		7 (398 ± 131)		20 (558 ± 63)	<0.05	42 (516 ± 58)	36
C		7 (466 ± 96)		23 (381 ± 35)	<0.05	47 (401 ± 35)	36
D		8 (332 ± 51)		21 (332 ± 80)	<0.05	43 (332 ± 42)	33
E		13 (473 ± 85)		10 (440 ± 96)	0.68	34 (458 ± 62)	32

dt, decision time (s).
P values are of binomial tests.
n = total spiders used, including those that made no choice.

Table 3. Stimuli used and attack rate of *Evarcha culicivora* in two-choice tests.

Experiment	Image 1	Pounced on image 1 (mean dt ± SEM)	Image 2	Pounced on image 2 (mean dt ± SEM)	*P*	Total *n* (mean dt ± SEM)	Attrition (%)
F		13 (187 ± 57)		11 (215 ± 74)	0.84	32 (200 ± 45)	31
G		20 (108 ± 34)		8 (375 ± 26)	<0.05	48 (185 ± 34)	42
H		20 (145 ± 26)		2 (496 ± 22)	<0.05	35 (177 ± 30)	37

dt, decision time (s).
P values are of binomial tests.
n = total spiders used, including those that made no choice.

one stimulus over the other (Table 3). There was no significant difference in the decision time between the two abstract images ($t = 0.307$, d.f. = 22, NS; experiment F, Table 3).

Comparing decision times in the two-choice experiment of *H. albovittatum* and of *E. culicivora*, we found significant differences in the decision times when faced with abstract images (Figure 1H–I), with *E. culicivora*'s decision time being significantly shorter than that of *H. albovittatum* (Table 2, experiment C and Table 3, experiment G: $U = 10.0$, d.f. = 2, $P = 0.001$; Table 2, experiment D and Table 3, experiment H: $U = 36.5$, d.f. = 2, $P < 0.05$). We found no difference ($t = 0.098$, d.f. = 29, NS) in decision times to choose a realistic image of a fly (Table 2, experiment C and Table 3, experiment G). When comparing experiments A and F (Tables 2 and 3), which have only abstract images (Figure 1H–I), the average decision time of *E. culicivora*, regardless of choice, was significantly shorter than that of *H. albovittatum* ($U = 134.0$, d.f. = 2, $P < 0.01$).

Comparing the attrition rates in the two-choice experiment of *H. albovittatum* and of *E. culicivora*, we found significant differences in the experiments that showed only abstract images (Fisher's exact test, Table 2, experiment A vs. Table 3, experiment F: $P < 0.001$), with the attrition rate of *E. culicivora* being significantly lower than that of *H. albovittatum.* We found no differences in the attrition rates between the salticid species in experiments that contained the realistic image of a fly (Fisher's exact test, Table 2, experiment C vs. Table 3, experiment G: $P = 0.675$; Table 2, experiment D vs. Table 3, experiment H: $P = 0.811$).

Discussion

These data show how the biological relevance of a stimulus is manifested in the processes of object recognition, with the specialist predator *E. culicivora* quickly recognising abstract images as its preferred prey through feature extraction of key elements, while the generalist predator *H. albovittatum* appears to use no such short-cut for classification. 'Implicit representation' is the use of key elements alone for creating a broad 'perceptual envelope' of

images categorised as prey (Ewert 2004; Prete et al. 2011; Nelson & Jackson 2012). This process would be primarily beneficial for generalist predators making a broad category classification (i.e. 'prey'). We previously showed that *E. culicivora* recognises the abstract images of *Anopheles* mosquitoes specifically as its preferred prey (Dolev & Nelson 2014). This highly specific type of classification could not be the result of a broad-based implicit representation; rather, it seems that *E. culicivora* uses a narrow perceptual envelope to classify the stimulus, or 'implicitly identifies' the images specifically as *Anopheles*.

The results of the multi-choice experiment suggest that *H. albovittatum* is a generalist predator. With our relatively low sample sizes we can only rule out a strong preference to a particular prey item. However, for the purposes of this study we can reasonably consider *H. albocittatum* a generalist, as it initiated stalking behaviour roughly equally to each of the six stimuli used, suggesting that it categorised all images as potential prey items. Although a few individuals chose the circle (roughly half the number that chose the other stimuli), it is not entirely surprising that the spiders categorise the circle as a prey item. Many generalist predators, including some salticids, categorise stimuli as prey using only basic key features (Drees 1952; Barlow 1953; Lettvin et al. 1959; Heinze et al. 1998; Prete et al. 2011; Bartos 2013). What is somewhat surprising was the high attrition rate in this experiment, as salticids typically readily respond to stimuli in the lab (Harland et al. 1999). The most likely explanation is that, rather than stalk distant prey, *H. albovittatum* tends to pounce on nearby prey. This suggestion is strengthened by the lower attrition rates in the two-choice experiments, where stimuli were presented at a distance from which spiders could directly pounce on the target. Indeed, in the two-choice tests where a realistic image was presented to *H. albovittatum*, the attrition rates were no different than those of *E. culicivora* from our previous work (Dolev & Nelson 2014). Interestingly, when the spiders were presented only with abstract images, *H. albovittatum*'s attrition rate increased significantly while *E. culicivora*'s remained unchanged.

Similarly, the decision time prior to an attack on the realistic and abstract images differed between the specialist and the generalist. Given the nature of the two-choice experiment, decision time can be considered as a proxy for how long it takes the spiders to visually analyse an image. Unsurprisingly, both species took the same amount of time to analyse the image of the fly. However, *E. culicivora* analysed the abstract images (including the scrambled version) significantly faster than *H. albovittatum,* and also significantly faster than they themselves analysed alternative stimuli.

Bednarski et al. (2012) show that the salticid *Phidippus audax* (Hentz, 1845) uses stimulus movement as the key element for categorisation as prey, thus adopting a very large perceptual envelope of this category (including a moving rectangle). In a similar set of studies, Bartos (2007, 2013) showed that the salticid *Yllenus arenarius* Menge, 1868 uses four key elements (stimulus length, movement type, congruent location of body parts and number of appendages) for the classification of a stimulus as prey. Furthermore, stimulus length and type of movement are used for classification into two prey categories with distinct escape risks requiring different attack strategies. These key elements create somewhat smaller perceptual envelopes than that of *P. audax*. Together with our data, these exemplify the differences in object categorisation and image analysis that seem to be the result of the evolution of predatory specialisation. One can imagine an evolutionary spectrum, starting with a generalist predator such as *P. audax*, which represents prey as anything that moves 'the right way'. As specialisation evolved, the use of more cues enables the

creation of narrower perceptual envelopes and the application of specific prey-catching behaviours, accordingly. At the other end of the spectrum, we find a highly specialised predator, such as *E. culicivora*, with a strong preference for one specific prey which it 'implicitly identifies' and which triggers prey-specific predatory behaviour.

Hypoblemum albovittatum and *E. culicivora* appear to classify the same images as different things. *Hypoblemum albovittatum*, being a generalist predator, did not appear to categorise the abstract images as prey items, while *E. culicivora* not only categorised them as prey, but identified them as preferred prey. *Evarcha culicivora*'s ability to recognise the abstract scrambled mosquito relies on feature abstraction (Dolev & Nelson 2014) and here we show that this recognition and categorisation process is performed more rapidly for abstract stimuli than for realistic stimuli. *Evarcha culicivora* also processed abstract images more rapidly than the generalist salticid. This suggests that the feature extraction processing is a benefit that coevolved with predatory specialisation in *E. culicivora.* The processing of the realistic images used in these tests took longer. It is therefore reasonable to assume that, compared with the quick low-level feature extraction characteristic of preferred prey recognition, analysing images that lack these features might require higher-level holistic processing.

Our work suggests that, compared with generalists, specialists may use streamlined categorisation processes that facilitate the rapid identification of stimuli relevant to their specific life histories. Indeed, one can envision that it is the very strength of the implicit representation strategy (basing prey recognition or representation on 'algorithms', rather than underlying neural components) that has paved the way for the evolution of predatory specialisation, either by narrowing the subset of objects that elicit appetitive behaviours, or by having some of these objects elicit a stronger appetitive behaviour.

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Appendix 6

ICN 2014 Poster

Following is a PDF version of my poster from the International Society for Neuroethology 2014 conference in Sapporo, Japan. This poster was awarded the first prize in the conference poster competition.

What Do You See?

Pattern recognition and categorisation in jumping spiders

Yinnon Dolev; Ximena J. Nelson
School of Biological Sciences, University of Canterbury, Christchurch, New Zealand

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

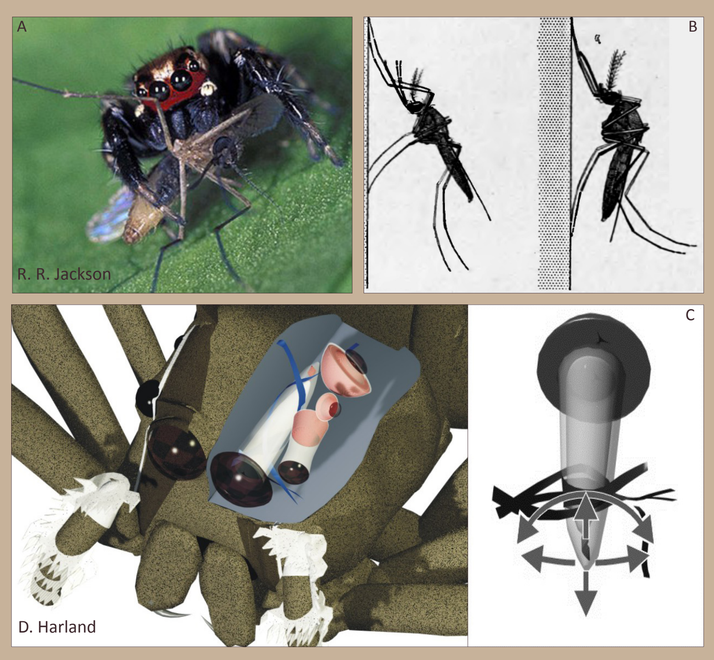
Introduction

Meet *Evarcha culicivora*, an East African jumping spider that feeds on human blood by preferentiality choosing blood fed female *Anopheles* mosquitoes as prey[1].

Like other jumping spiders[2], *E. culicivora* relies primarily on vision for locating, recognising and hunting its prey, using its large forward facing eyes, and movable retinae to analyse its world.

E. culicivora uses the distinct resting posture and engorged abdomen characteristic of *Anopheles* as key elements for their recognition[3], however, the underlying perceptual processes are unknown.

Here, for the first time in spiders, we investigated perceptual classification of objects using *E. culicivora*'s predatory behaviour towards abstract stimuli.



A) Male *E. culicivora* feeding on a mosquito.

B) Typical resting postures of *Anopheles* (left) and *Culex/Aedes* (right) mosqitoes[4].

C) Salticid eye tubes depicted within the carpace (left) and movment degrees of freedom (right)[5].

Hypotheses

***E. culicivora* will:**

1) stalk and pounce on simplified representation of prey.

2) preferentially choose a simplified representation of its preferred prey over realistic non-preferred prey.

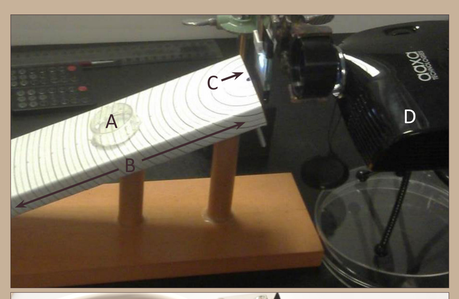
Methods

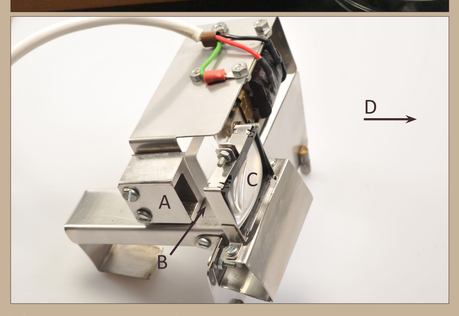
Is it food?

Single-stimulus predatory behaviour experiments testing the spider's classification of the different stimuli

Is it good food?

Two-choice experiments testing the spider's prey preferences





The experimental apparatuses.
A) Starting position; B) Ramp;
C) Focusing screen; D) Projector

Results

Even when scattered elements of the stimuli were disconnected, spiders categorised the abstract images as prey items (Table 1), preferentially choosing them over non-preferred prey (Table 2), but surprisingly, altering the inter-element angles rendered the perception of the stimulus as no longer relevant.

Table 1: Responses of the spiders to the different stimuli. Crosswise comparisons of the stalking responses to the different stimuli, using McNemar tests with a binomial distribution and Cochrans Q test.

Stimulus							
Stalked N>26	74%	64%	74%	77%	56%	33%	24
	-	NS	NS	NS	NS	<0.001	<0.001
	-	-	NS	NS	NS	<0.05	<0.005
	-	-	-	NS	NS	<0.05	<0.001
	-	-	-	-	NS	<0.005	<0.001
	-	-	-	-	-	NS	<0.05
	-	-	-	-	-	-	NS

Table 2: Binomial test results of two-choice predatory behaviour experiment stimulus pairs (N > 21).

Image 1		Image 2	Chose Image 2	p
	>		9%	<0.001
	>		25%	<0.05
	>		29%	<0.05
	>		19%	<0.05
	≈		46%	0.84

Conclusions

E. culicivora makes discriminations based on abstract concepts, including hypothetical angles formed by discontinuous elements.

It is not merely the resting posture[3], but the inter-element angle that is important for correct identification of *Anopheles.*

Our results[6] provide a glimpse of the underlying processes of object recognition in animals with minute brains, and suggest that these spiders use local processing for object recognition, rather than holistic or global processes.

References and Acknowledgments

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